
SHOUT
SHARE
IT

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

Editorial Staff.

E. G. Conklin—*The University of Pennsylvania.*

Jacques Loeb—*The University of California.*

T. H. Morgan—*Columbia University.*

W. M. Wheeler—*American Museum of Natural History, New York.*

C. O. Whitman—*The University of Chicago.*

E. B. Wilson—*Columbia University.*

Managing Editor.

Frank R. Lillie—*The University of Chicago.*

Volume VII

WOODS HOLL, MASS.
JUNE, 1904, TO NOVEMBER, 1904.

869

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA

CONTENTS OF VOL. VII.

No. 1. June, 1904.

PAGE

ARTHUR W. GREELEY..... 1

ARTHUR W. GREELEY: *Experiments on the Physical Structure of the Protoplasm of Paramœcium and its Relation to the Reactions of the Organism to Thermal, Chemical and Electrical Stimuli*..... 3

FRANK R. LILLIE: *Experimental Studies on the Development of Organs in the Embryo of the Fowl (Gallus domesticus)*... 33

W. K. GREGORY: *The Relations of the Anterior Visceral Arches to the Chondrocranium*..... 55

EVERETT F. PHILLIPS: *Variation in Bees: A Reply to Mr. Lutz.* 70

L. R. CARY: *Notes on a Peculiar Actinozoan Larva*..... 75

No. 2. July, 1904.

J. E. DUERDEN: *The Morphology of the Madreporaria, V. Septal Sequence* 79

BASHFORD DEAN: *Evolution in a Determinate Line as Illustrated by the Egg-cases of Chimæroid Fishes*..... 105

WILLIAM PATTEN: *New Facts Concerning Bothriolepis*..... 113

No. 3. August, 1904.

C. M. CHILD: *Form-Regulation in Cerianthus, V*..... 127

ALICE M. BORING: *Closure of Longitudinally Split Tubularian Stems* 154

T. H. MORGAN AND A. E. SCHIEDT: *Regeneration in the Planarian Phagocata gracilis*..... 160

ESTHER F. BYRNES: *On the Skeleton of Regenerated Anterior Limbs in the Frog*..... 166

ADELE M. FIELDE: *Observations on Ants in their Relation to Temperature and to Submergence*..... 170

No. 4. September, 1904.

S. W. WILLISTON: *The Temporal Arches of the Reptilia*..... 175

C. M. CHILD: *Form-Regulation in Cerianthus, VI*..... 193

ADELE M. FIELDE: *Portable Ant-Nests*..... 215

Edwin G. Conklin: *Experiments on the Origin of the Cleavage Centrosomes* 211

No. 5. October, 1904.

Adele M. Fielde: *Power of Recognition Among Ants* 227
Chas. W. Hargitt: *Notes on a Hitherto Undescribed Hydroid from Long Island Sound* 251
Edwin C. Starks: *A Synopsis of Character of some Fishes Belonging to the Order Haplomi* 254

No. 6. November, 1904.

C. M. Child: *Form-Regulation in Cerianthus, VII* 263
Charles D. Snyder: *Locomotion in Batrachoseps with Severed Nerve-cord* 280
Alexander G. Ruthven: *Butler's Garter Snake* 289
Adele M. Fielde: *Tenacity of Life in Ants* 300



ARTHUR W. GREELY.

Vol. VII. *June, 1904.* *No. 1*

BIOLOGICAL BULLETIN

ARTHUR W. GREELEY.

Arthur W. Greeley died at St. Louis, after an operation for appendicitis, on March 15, 1904, at the age of twenty-eight. The following paper, which he had prepared for publication just before his death, will indicate how lamentable for American science is the loss of this enthusiastic, industrious and keen investigator.

In this suggestive paper, explaining on the basis of modern physical chemistry the changes in structure of protoplasm accompanying changes in function, Greeley has mapped out for others work which he had planned for himself, but which he was unable to accomplish. There has been hitherto no thorough study of the changes in structure of living protoplasm produced by salts from the standpoint of modern views of electrolytes and colloidal solutions. This paper is a most important contribution to this subject and opens up a great field for further work. It was Greeley's good fortune to be able to reduce the changes to order, and thus to supply the structural basis for observed changes in function produced by salts and other agencies. As a result of this work he was able to reduce many of the so-called "tropic" responses of organisms to a common basis; all agencies producing a certain change in the protoplasm producing also a definite response in orientation of the organism. What a great step in advance this is will be appreciated by those familiar with the confusion prevailing in this most difficult field.

This paper, in connection with his earlier discoveries of the production of spores in infusoria by cold; on the identity of the physiological action of dehydration and exposure to low temperatures, and of the production of artificial parthenogenesis in the echinoderm egg by cold, stamps Greeley as an original,

industrious and accurate investigator of great scientific ability, doing work of a most fundamental character. All of those who knew him feel that in his death a man of great promise has passed away.

Dr. Greeley was born in Oswego, New York State, in 1875. He took his undergraduate degree in Stanford University in 1898, and spent one year as a graduate student in zoölogy, during which he went to Alaska with the fur-seal expedition and to Brazil with the Agassiz expedition. The following year he was a teacher in the State Normal School at San Diego, leaving there to enter the University of Chicago as fellow in physiology. Two years later he took his doctorate of philosophy under Loeb with a thesis on the action of low temperatures on the infusoria, and was then appointed Assistant Professor of Zoölogy at the Washington University, in St. Louis. For three summers he was a member of the staff of instruction in physiology at the Marine Biological Laboratory of Wood's Holl. During his two years of residence in St. Louis his enthusiasm and unusual personality had already aroused marked interest in biological science in that city.

Dr. Greeley was of a rare and winning personality, remarkable for extraordinary enthusiasm which inspired all with whom he came in contact. He had a happy disposition, great courage and high principles. His frank open nature, his consideration for others and his loyalty made him many friends ; and he had no enemies. He was an inspiring teacher. To his university, to his friends and to his colleagues his sad death at the outset of a most promising career is an irreparable loss.

A. P. Mathews.

EXPERIMENTS ON THE PHYSICAL STUCTURE OF THE PROTOPLASM OF PARAMŒCIUM AND ITS RELATION TO THE REACTIONS OF THE ORGANISM TO THERMAL, CHEMICAL AND ELECTRICAL STIMULI.

ARTHUR W. GREELEY.

CONTENTS.

PAGE.
Introduction. 3
Structural Reactions of the Protoplasm of Protozoa to Physical and Chemical Changes.
I. Reactions to Variations in the Temperature. 8
II. Reactions to Chemical Changes. 9
III. Reactions to the Electrical Current. 19
Effect of these Structural Modifications on the Vital Properties of the Protoplasm.
I. Growth and Cell Division. 22
II. The Tropisms. 24
1. Thermotaxis. 24
2. Galvanotaxis. 25
3. Chemotaxis. 29
Conclusions. 31

INTRODUCTION.

It has been known for several years that a marked similarity in physical structure exists between protoplasm and that class of chemical solutions known as colloidal solutions. This similarity was pointed out by Hardy in 1899 as a result of his investigations upon the physical structure of certain organic colloids, as egg albumen, gelatin, etc., which resemble protoplasm very closely in their gross appearance, and of observations upon protoplasm itself under various conditions.

The conclusions of Hardy and others[1] in regard to the physical structure of the organic colloids are of such importance in the further development of this paper, and are so largely unappreciated by biologists, that they will be briefly recapitulated at this place.

1. A colloidal solution consists of a fluid matrix which holds in suspension more solid or viscous granules (the colloidal par-

[1] Hardy, *Jour. of Physiol.*, 1899, XXIV., p. 172; *ibid.*, p. 288. Mann, "Physiological Histology," Oxford, 1902.

ticles), which differ from the dissolved substance in a crystalloidal solution in that they are relatively very large aggregates of molecules of the colloidal matter. These particles do not affect the osmotic pressure of the fluid matrix; while in a crystalloidal solution the solute exists in a molecular or ionic condition, and thus gives to the solution a definite osmotic pressure.

2. The physical state of the entire solution depends on the condition of these colloidal particles. When they are finely divided and separated from each other in the solvent, the colloid appears as a liquid or exists in the "sol" phase. If, however, the colloidal particles become fused, and thus lose their condition of fine suspension, the colloid becomes relatively solid, or passes into the "gel" phase.

3. The physical state of the colloidal particles and hence of the entire solution varies directly with certain external conditions. Thus the passage from the "sol" into the "gel" phase is accomplished in the following ways: (*A*) by variations in the temperature, (*B*) by chemical changes, (*C*) by the action of the electric current.

A. The physical state of the organic colloids varies directly with the temperature. If the "sol" phase is constant at the normal temperature, 20° C., coagulation gradually takes place as the temperature is lowered, until at 0° C. almost complete gelation has occurred. As the temperature is raised above the normal the fluidity of the solution is increased (by the subdivision of the colloidal particles and absorption of water), until a critical point is reached at which coagulation suddenly occurs, and the colloid goes into a condition of heat rigor.

B. A colloid may be coagulated by adding to it a solution of any electrolyte which bears an electrical charge opposite in sign to that carried by the colloidal particles. Thus Hardy found that a positively charged colloidal solution was coagulated by any electrolyte with a powerful anion or negatively charged ion, and the rapidity of the coagulation varied directly with the valence of the anion. A negatively charged colloid was coagulated by electrolytes of an opposite electrical character, or by powerful cathions. Similarly an electrolyte carrying a charge of the same sign as that of the colloidal particles causes them to subdivide

still further. As a result water is absorbed so that a liquefaction of the whole colloid occurs, as by a slight increase in the temperature. Thus cathions liquefy positively charged colloids; anions, negatively charged colloids.

C. The same conditions prevail in the reaction of colloids to the electrical current. Negatively charged colloidal particles fuse and form a "gel" around the anode, and tend to liquefy about the cathode during the passage of the current. Positively charged colloids coagulate about the cathode, and liquefy about the anode.

From the behavior of these colloidal solutions under various chemical and electrical conditions Hardy concluded, that the "sol" phase is maintained under normal conditions because all the colloidal particles carry an electrical charge of the same sign, and are thus mutually repelled, and remain in a state of fine suspension. Whenever there is introduced an electrical charge of an opposite sign, either by means of a dissociated electrolyte or by the electrical current, the charge carried by the colloidal particles is neutralized, and fusion or coagulation occurs.

The reaction to temperature variations is apparently due to the fact that, within certain limits, the kinetic energy of the colloidal particles varies directly with the temperature. A reduction of the kinetic energy causes a gradual fusion of the particles; an increase brings about a still finer state of suspension, and consequent liquefaction of the colloid through the absorption of water. The sudden coagulation at the critical point is probably due to a chemical change in the colloid itself.

The chief points of similarity between these colloidal solutions and protoplasm made apparent by Hardy's work are as follows:

1. The elementary physical structure of the two is the same. Like the colloid, protoplasm is known to be made up of two substances — (*a*) the fluid cell-sap or matrix which holds in suspension (*b*) the more solid proteid or protoplasmic particles, granules or microsomes of the morphologists. And the physical state of the protoplasm depends on the condition of these protoplasmic granules, just as the state of the artificial colloid depends

on the condition of its constituent particles. It is significant to find, that all the controversies that have been waged over the various theories of a fixed morphological structure in protoplasm have centered about the supposed unchanging physical condition of these more solid or viscous elements in the protoplasm. Now in the artificial colloids we see that their physical state varies directly with certain external conditions. The protoplasmic particles of course are vastly larger than the particles in an artificial colloidal solution, and we have no right to assume that the two are identical. But the protoplasmic granules bear the same relation to the cell-sap as the colloidal particles do to the fluid matrix, and they both respond to chemical and physical changes in a similar manner. A close comparison of the behavior of these two structural elements under various conditions will, I believe, throw a great deal of light on the physical basis of protoplasm.

Hardy observed that when a colloidal solution is exposed to the action of certain so-called fixing agents, the same structures are produced as by the action of these fixing agents upon protoplasm, *viz.*: a coagulation occurs in which the colloidal particles fuse in definite ways, producing a type of structure varying with the fixing agent employed. He therefore concluded that coagulation of the protoplasm is necessary to reveal the fixed types of structure thought by some to be characteristic of protoplasm under all conditions. Such types of structure then appeared to be in protoplasm, as in the organic colloids, merely artefacts; and Hardy argued that protoplasm in the living condition must be identical, as far as its physical structure is concerned, with the organic colloids in the "sol" phase, and that like the colloids its structure is constantly changing with the external conditions. Undoubtedly under the same external conditions, the protoplasm of different forms will show varying types of structure, as the above statement will allow. In some cases this structure may resemble a reticulum or other types described by the morphologists, but no one will now maintain that such a type is of universal occurrence. In the living protoplasm of *Paramœcium*, I have never seen a trace of a fixed structure.

These suggestions of Hardy and others upon the physical structure of protoplasm remained almost unnoticed by biolo-

gists until, very recently, attention was directed to them by the work of Loeb,[1] Mathews,[2] R. S. Lillie and others. Mathews, in particular, in his work upon the chemical stimulation of the motor nerve of the frog, has arrived at the conclusion that the protoplasm of the nerve is a colloidal solution whose particles carry a definite electrical charge, and that stimulation of the nerve is accomplished by a reversible change in the physical state of the protoplasm, analogous to coagulation. He finds that this stimulation or coagulation is effected chemically only by a series of electrolytes which agree in carrying a predominant negative charge of electricity, as would be the case in Hardy's positively charged colloidal solutions. It is only fair to say that these remarkable investigations of Mathews have been the inspiration of this work on the physical structure of protoplasm. R. S. Lillie[3] shows, in his work on the reaction of cytoplasmic and nuclear structures to the electric current, that these forms of protoplasm behave exactly as would positively and negatively charged colloidal solutions under the same conditions.

The experiments described above have shown that protoplasm reacts to various external conditions in a manner strictly parallel to the behavior of colloidal solutions in the presence of like stimuli. But meanwhile we have had but little evidence of the precise structural changes, that accompany these reactions, and are, as we have seen, the leading distinguishing feature of colloidal solutions. It was with the idea of studying these structural changes, which occur in protoplasm when subjected to various external stimuli, and of comparing them with the changes in colloidal solutions under the same conditions, that the following experiments were undertaken.

The material used has been the protoplasm of various protozoa, viz: *Paramœcium*, *Stentor*, *Amœba* and others, which are ideal objects for this study because of the abundance in which

[1] Loeb, as a result of his work on the stimulation of contractile tissue by ions, and on the toxic and antitoxic effect of ions on the duration of life of the *Fundulus* egg and frog's muscle, was the first to suggest, that the protoplasm reacts under these conditions like artificial colloids, although he has elsewhere adopted a different explanation. (See *Amer. Jour. Physiol.*, 1902, VI., p. 411.)

[2] Mathews, *Science*, 1902, XV., p. 492; 1903, XVII., p. 729.

[3] Lillie, *Amer. Jour. Physiol.*, 1903, VIII., p. 273.

they can be procured, and especially because their small size makes it possible to study the structural changes in the living protoplasm under high magnifications, which is out of the ques-question in more bulky tissues. The protozoa were subjected to thermal, chemical and electrical changes, and the structural modifications produced by these means were studied.

Structural Reactions of the Protoplasm of Protozoa to Physical and Chemical Changes.

I. *Reactions to Variations in the Temperature.*

In previous papers[1] I have described the structural changes that occur in the protoplasm of various protozoa when exposed to variations in the temperature, so that a very brief description will suffice here. We find, exactly as in the case of organic colloids, that the fluidity of the protoplasm varies directly with the temperature within certain limits. As the temperature is lowered below the normal, 20°C., a very gradual coagulation occurs, because of the decrease in kinetic energy and consequent fusion of the protoplasmic particles. This change is accompanied by a loss of water, so that at 0° C. there is produced a nearly solid opaque, spherical mass of protoplasm, which exists only in a resting condition. As the temperature is elevated above the normal, the reverse changes occur through the increase in kinetic energy acquired by the protoplasmic particles. They may be seen to subdivide further and become more widely separated through the absorption of water, so that the fluidity and motility of the protoplasm is greatly increased. These changes continue until the critical point is reached, at which coagulation suddenly occurs, and the protoplasm goes into heat rigor, probably because of some chemical change in the protoplasm itself. So closely do these structural changes agree with those that occur in artificial colloids under the same conditions, that a description of one would apply equally well for the other. Indeed, it was this striking similarity between the results in the two cases, that led me to compare the reactions of colloids and the protoplasm of

[1] Greeley, *Amer. Jour. Physiol.*, 1901, VI., p. 201; Biol. Bull., 1902, III., p. 165; *ibid.*, 1903, V., p. 42.

various Protozoa to chemical and electrical stimuli to see what light these reactions might throw on the physical structure of protoplasm.

II. *Reactions of the Protoplasm of Paramœcium and other Protozoa to Chemical Changes.*

Paramœcia reared on cultures containing bread were used chiefly in these experiments, although other protozoa were utilized for purposes of comparison. The method employed in the experiments was as follows: The solutions whose action upon the protoplasm was to be tested were made up in the proper dilutions, and distributed among Minot watch glasses holding about 10 c.c. of the solution. Pure, concentrated cultures of the protozoa were obtained, and a drop or two added to each of the dishes containing the solutions. At short intervals the protozoa were examined to observe any structural changes in the protoplasm. A 1/12 inch oil immersion was used to make out the finer details.

Great care is needed to distinguish between chemical and osmotic effects. In order to obviate the possibility of modifying the protoplasm through the extraction of water, all the solutions were used in concentrations of a lower osmotic pressure than that of the protoplasm, roughly equal to a $m/40$–$m/50$ cane sugar solution. In some cases the dilutions were very great, and, inasmuch as distilled water or any solution of a lower osmotic pressure will liquefy the protoplasm through the absorption of water osmotically, there would be danger of confusing the osmotic and chemical effects of the solutions, were it not for the fact that osmotic and chemical liquefaction can be readily distinguished by the type of protoplasmic structure produced by each means.[1]

All the chemicals used fall into two classes:

1. Those that effect the protoplasm only through the osmotic pressure of the solution.

[1] Miss Towle, in investigations now in progress at the University of Chicago, has observed that paramœcia may live indefinitely in redistilled water. It is probable that in this case, as in others mentioned in this paper, the variations in the structural reactions are due to the differences in the chemical composition of the cultures in which the paramœcia were reared. But this question of the structural effect of distilled water requires further investigation.

2. Those that modify the structure of the protoplasm independently of the osmotic pressure. The first class includes the non-electrolytes used, the second, the electrolytes.

Non-electrolytes. — The non-electrolytes used, distilled water, cane sugar and urea, affect the protoplasm of paramœcia only through the osmotic pressure of the solution. There is no chemical effect. All those solutions hypotonic to protoplasm produce a liquefaction through the absorption of water, while isotonic solutions have no effect on the structure of the protoplasm, and hypertonic solutions coagulate the protoplasm through a withdrawal of water. These effects are shown in the following table:

TABLE I.

	m/5	*m*/10	*m*/40	*m*/160
Cane sugar,	Coagulation.	Coagulation.	No effect.	Liquefaction.
Urea,	"	"	"	"

An examination of these structural changes under a high magnification makes clear the fact that these non-electrolytes modify the structure of the protoplasm solely through a change in the amount of water in the fluid matrix which holds the more viscous elements in suspension. The size of the protoplasmic particles remains unaffected. In hypotonic solutions the protoplasmic particles are more widely separated by an increase in the amount of water in the fluid matrix. In hypertonic solutions they are brought closer together, or may fuse through a withdrawal of the surrounding liquid.

Electrolytes. — As far as their effect on the physical structure of the protoplasm of *Paramœcium* is concerned, all the electrolytes used fall into two classes: first, those that coagulate even in solutions whose osmotic pressure is far less than that of the protoplasm; second, those that liquefy the protoplasm in any dilution. Moreover, all the members of the first class agree as far as their electrical conditions are concerned. They all have a predominantly powerful cathion,[1] but resemble each other in no other particular. Likewise all the members of the second class agree in possessing a predominantly powerful anion. Thus we

[1] This "predominance" may be a function of the solution tension of the ions. See Mathews, *Amer. Jour. of Physiol.*, 1904, X., p. 290.

see that anions, or negatively charged ions, liquefy the protoplasm of *Paramœcium*, cathions, or positively charged ions, coagulate without any regard for the supposed chemical affinities of the electrolyte.

That we are not dealing with a specific chemical effect for each electrolyte is still further shown by the fact that the activity of each solution is roughly proportional to the valance of the predominant ion. Thus salts containing trivalent anions or cathions are effective in much greater dilutions than bivalent or univalent salts. All these facts are indicated in the accompanying table which gives a list of the electrolytes used, their effects on the physical structure of the protoplasm, and the greatest dilution at which they are effective. The maximal dilutions can be only approximate, as the action of identical solutions is not the same on paramœcia from different cultures, because no two are exactly alike in respect to chemical composition and osmotic pressure. Especially is this true of the liquefying electrolytes, for, as we have seen, in very dilute solutions it is frequently difficult to determine the point at which the specific liquefying action of the anion ends and the osmotic absorption of water commences. As has been already mentioned, there are structural differences between these two forms of liquefaction which enable us to distinguish between them with considerable accuracy, and the fact of chemical liquefaction can be easily demonstrated by using the liquefying substances in solutions about isotonic with protoplasm. Of course no such difficulty arises with the coagulating solutions, for at great dilutions the specific coagulating action of the cathion must overcome the tendency for the protoplasm to be liquefied through the entrance of water osmotically, and there is thus a well-defined point at which the coagulation of the protoplasm by the cathion ends and its osmotic liquefaction commences.

TABLE II.

Coagulating Solutions.	Liquefying Solutions.
$m/1{,}600$ HCl, $m/1{,}600$ HNO_3	$m/1{,}600$ NaOH, $m/1{,}600$ KOH
$m/2{,}400$ H_2SO_4	$m/1{,}600$ $Ba(OH)_2$, $m/1{,}600$ $Sr(OH)_2$
$m/40$ KCl	$m/40$ NaCl, $m/40$ NH_4Cl, $m/40$ $NaNO_3$
$m/640$ $MgCl_2$, $m/640$ $CaCl_2$, $m/640$ $Ca(NO_3)_2$, $m/640$ $BaCl_2$	$m/640$ Na_2SO_4, $m/640$ $(NH_4)_2C_2O_4$
$m/320$ $MgSO_4$	$m/2{,}400$ Na_3PO_4, $m/2{,}400$ $Na_3C_6H_5O_7$
$m/1{,}600$ Al_2Cl_6, $m/1{,}600$ Fe_2Cl_6	

It will be seen by an examination of the table that the activity of any of the salts, as is shown by the strength of solution required to modify the structure of the protoplasm of *Paramœcium*, varies directly with their valence. The acids and bases are much more powerful in their action than any of the salts of a similar valence, probably because of the known disproportionate kinetic energy of the hydrogen and hydroxyl ions.

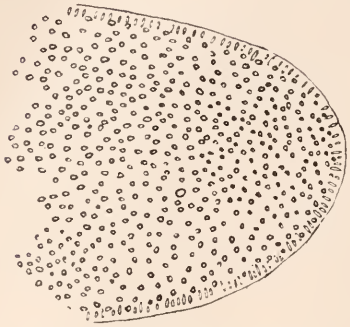


FIG. 1. Normal protoplasm of *Paramœcium*. $\frac{1}{12}$ in. oil immersion.

All the acids and salts found in the first column of the table, which agree in coagulating the protoplasm through the action of the predominant cathion, effect changes in the protoplasm so similar as to be practically indistinguishable even under a high magnification. The less active solutions, such as KCl and $MgSO_4$, do not produce quite so dense a coagulum as the others, and the reaction is considerably slower. But in all the bivalent and trivalent salts and the acids, a distinct clouding of the protoplasm can be observed within thirty minutes after the paramœcia have been immersed in the solution. This clouding of the protoplasm increases and is accompanied by a shrinking of the cell owing to a loss of water, until within a few hours, the whole cell is reduced to a subspherical mass of densely opaque protoplasm (see Fig. 2). The changes are identical with those produced by a lowering of the temperature.

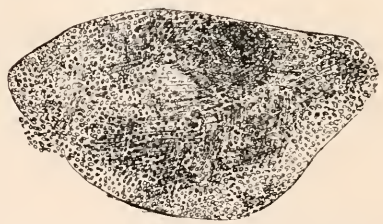


FIG. 2. A *Paramœcium* in $m/320$ $MgCl_2$, showing a typical coagulation of the protoplasm.

An examination of the protoplasm with a one-twelfth inch oil-immersion lens reveals the fact that the clouding of the protoplasm is due to a separation of the two elements of the protoplasm, the cell sap and the protoplasmic granules. These two elements lose

their state of fine mixture or suspension, which is always characterisic of motile protoplasm, and the protoplasmic particles tend to fuse into a spongy coagulum or "gel" from which the cell sap continually escapes, until a complete separation of the two elements is brought about. This fusion of the protoplasmic particles may result in the formation of two varieties of coagulated structure. The fused particles may appear as spherical bodies of proteid material, unconnected but closely massed together in such a way as to form an exceedingly dense coagulum



FIG. 3. Coagulated protoplasm of *Paramœcium* under $\frac{1}{12}$ oil-immersion. A dense coagulum formed after an exposure to *m*/320 $CaCl_2$ for twenty-four hours.

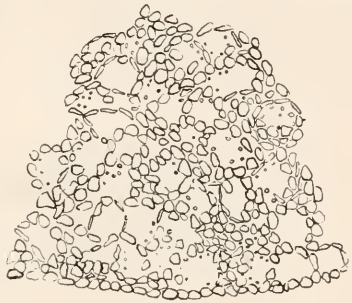


FIG. 4. Another type of coagulation viewed under the $\frac{1}{12}$ oil-immersion. The result of a ten hour residence in *m*/800 HCl, showing a trace of the reticular structure.

(see Fig. 3). Or the protoplasmic particles may fuse into fibrils or anastomosing threads which form an incomplete network, holding the cell sap in its interstices. The fibrils may eventually become thicker and transform the network into a relatively solid coagulum (Fig. 4). These last forms of coagulated structure are very similar to the network formations obtained by Hardy in organic colloids and various protoplasmic tissues by the action of fixing agents, and have been formerly supposed to be characteristic of living protoplasm. As in Hardy's experiments these fibrillar or reticular structures never appear in the living protoplasm of *Paramœcium*, but are solely an incidental result of the process of coagulation by chemical or other means.

The salts and bases in the second column of the table, which produce a common liquefaction of the protoplasm of *Paramœcium* through the action of a predominant anion, are effective like the

coagulating solutions, in dilutions which are roughly proportional to their valence.

The univalent salts have a comparatively weak effect upon the protoplasm, and relatively high concentrations are necessary before we can be sure that the liquefaction is due to chemical and not osmotic means. In solutions of the bases, bivalent and trivalent salts, however, the effects are unmistakable, and are exactly the reverse of those initiated by the coagulating solutions. Within a very few minutes after immersion in the solution liquefaction first becomes discernible as a clearing of the protoplasm. This process proceeds until the protoplasm loses its characteristically granular appearance, and becomes semi-transparent. At



FIG. 5. A *Paramœcium* in *m*/320 Na_2SO_4, showing a typical liquefaction of the protoplasm.

the same time the cell membrane becomes greatly swollen through the absorption of water, which frequently gives the protoplasm a vacuolated appearance, and gives rise to droplets which cling to the protoplasm underneath the cell wall. The result is an irregular watery mass of protoplasm from which the solid elements have, to a superficial examination, completely disappeared (see Fig. 5). In the solutions of trivalent salts these changes occur with such violence that the cell membrane is disrupted, and the disintegrated protoplasm becomes scattered throughout the solution. Thus the liquefying anion has the same effect upon the protoplasm as a slight increase in temperature.

If, as in the case of the process of coagulation, these microscopic changes be studied under a one-twelfth inch oil-immersion, it will be at once seen that a change in the physical state of the protoplasmic particles is responsible for these profound structural modifications. The particles apparently continue to divide as the process goes on, until their size becomes so small that they

are discernible only under high magnifications. At the same time a rapid absorption of water takes place, and the particles become widely separated in the now exceedingly fluid cell sap. We thus have a means of distinguishing between liquefaction by chemical and osmotic means. As has been described above, the central feature of the process of chemical liquefaction is a splitting up of the protoplasmic particles and consequent imbibition of water through this increase in the absorbing surface (see Fig. 6). In the process of osmotic liquefaction, however, the size of protoplasmic particles remains unchanged, and water enters the protoplasm solely because of the osmotic relations between the cell-sap and the surrounding liquid. These two processes can be distinguished microscopically as indicated even under a low magnification, for paramœcia that are liquefied osmotically never lose their granular appearance, while those liquefied chemically become markedly transparent.

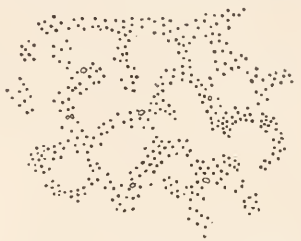


FIG. 6. Liquefied protoplasm of *Paramœcium* under $\frac{1}{12}$ oil-immersion. Formed by an exposure of two hours to $m/320$ Na_2SO_4.

That in these phenomena we are dealing also with variations in the surface tension relations of the protoplasm is apparent from the change of form which occurs during coagulation and liquefaction. The surface tension force is neutralized either by an increase in temperature or by giving all the protoplasmic particles a like charge which tends to make them mutually repellent, and thus introduces a disrupting force. Hence, if we destroy the electrical charge, we not only allow the protoplasmic particles to fuse, but we increase the surface tension. Thus, during coagulation, the cell tends to assume a spherical form which is characteristic of all resting cells. The opposite is true during liquefaction. The disrupting force is still further increased by the introduction of a charge of the same sign as that carried by the protoplasmic particles, and the cell assumes an irregular form. The most powerful anions used, the phosphate and citrate, bring about this disruption of the cell with almost explosive violence, so that the cell membrane bursts and the protoplasmic

particles are widely scattered. After a residence in these solutions of ten to fifteen minutes, no vestige of the paramœcia remains beyond the scattered protoplasmic granules.

The above experiments were repeated on *Vorticella*, *Stentor* and *Hydra*, and essentially the same structural changes were produced. The protoplasm of *Vorticella* reacts to electrolytes exactly as does the protoplasm of *Paramœcium*. In *Stentor* the differentiation between the two layers of protoplasm, endosarc and ectosarc, is more complete, and the less differentiated granular endosarc responds to the solutions in the typical manner, while the clear striated ectosarc remains practically unaffected. Thus the process of coagulation results in the formation of a cell whose endosarc is shrunken into a dense, spherical mass within the ectosarc, which retains its original form and size. During liquefaction the endosarc becomes wholly transparent, and we have produced an apparently empty cell which is bounded by the unchanged ectosarc as before. *Hydra* reacts in the same manner, the endoderm responding to the solutions as did the endosarc of *Stentor*; and we have produced animals of the original size and shape, but with either a coagulated or liquefied interior. It appears from these experiments that the physical conditions already described are applicable to protoplasm only in its primitive granular condition. The differentiation into such simple structures as the ectosarc of *Stentor*, or the ectoderm of *Hydra* has changed to some extent its elementary physical state. The high degree of viscosity of such protoplasm suggests that it is normally much nearer the "gel" phase.

Like all the typical physical modifications of protoplasm, these changes of structure are reversible, if they are stopped at the proper time. Thus coagulated protoplasm may be again liquefied by the action of a powerful anion, or liquefied protoplasm may be coagulated by a cathion. This fact seems to show with special force that the physical state of the protoplasm at any moment is the result of a definite reaction to the chemical conditions of the environment.

The whole behavior of protoplasm under the action of dilute solutions of electrolytes suggests the conclusion, that, as far as its chemical and physical reactions are concerned, it is a colloidal

solution whose particles carry a definite electrical charge. The colloidal particles in an artificial colloidal solution are apparently identical, as far as their physical reactions are concerned, with the protoplasmic or proteid particles held in the cell sap of protoplasm; at least they both react to thermal and chemical changes in the same way. The reaction to chemicals further demonstrates the fact, that, like the colloidal particles, these protoplasmic elements carry a uniform electrical charge under normal conditions; for by no other assumption can we explain the facts that salts, acids and bases modify the structure of protoplasm only by virtue of their electrical properties, and that non-electrolytes have no effect whatever beyond that of the osmotic pressure of the solution. Indeed the action is not, properly speaking, a chemical one at all, but an electrical one. In *Paramœcium* the protoplasmic particles are apparently negatively charged, and the mutual repulsion of these similarly charged particles, keeps them in a state of fine suspension. Under these conditions the protoplasm exists in a "sol" or liquid phase. Predominant cathions neutralize this negative charge; the repelling force is removed, and the particles fuse, forming a coagulum. A predominant anion further increases the disrupting force, and a state of still greater fluidity or liquefaction results.

A still closer comparison between artificial colloids and the protoplasm of *Paramœcium* is possible. It will be remembered that Hardy succeeded in reversing the charge carried by the colloidal particles by changing the chemical conditions of the solution. Thus the addition to the solution of a small amount of a base gave an alkali-modified colloid whose particles were negatively charged. Such a solution was coagulated only by cathions. Likewise the addition of a small amount of acid gave an acid-modified solution, whose particles were positively charged; this solution was coagulated by anions. How this change is effected by acids and alkalis we cannot say, but certain it is that the electrical nature of the colloid is determined by certain ions present in its fluid matrix.

The reactions of the protoplasm of paramœcia to electrolytes described above, occurred in organisms which had been reared in a normal, alkaline culture. It was found that when the culture

medium became acid through the fermentation of bread, the structural reactions of the protoplasm to electrolytes was changed. And they were changed in such a way as to suggest that there had been a reversal of the electrical charge carried by the protoplasmic particles, or in other words, that there had been formed an acid-modified protoplasm. Thus the protoplasm of paramœcia from an acid culture was not coagulated by cathions and liquefied by anions, as was the case without exception with paramœcia from an alkaline culture, but the reactions were as follows: The first change produced by the neutralization of the normal alkalinity of the culture was an irregularity in the structural reactions. Instead of all the organisms being coagulated by cathions and liquefied by anions, some were coagulated, and some were liquefied in each solution, apparently indicating a condition in which the protoplasm of some of the paramœcia had been modified by the acid, the rest being still unaffected. As the acidity of the solution increased, the number that were coagulated by anions and liquefied by cathions correspondingly increased until, in a few instances, a complete reversal of the structural reaction to electrolytes was produced. While the reversal was more often not complete, in all cases it occurred in a varying proportion of the paramœcia from acid cultures, while the remainder were generally rendered indifferent to the solutions which formerly coagulated or liquefied them. The complete reversal occurred only in the salt solutions. The acids always coagulated the protoplasm regardless of the character of the culture, and to alkalis, the paramœcia from acid cultures were only rendered indifferent. All these results indicate that the particles of the acid-modified protoplasm have become positively charged like the particles in Hardy's acid-modified colloids.

The view that this change in the structural reactions of paramœcia is due to a modification of the protoplasm by acids is rendered more probable if we follow the reactions of paramœcia from day to day, which are taken from a culture which is gradually becoming acid by the fermentation of bread. In such a case we can easily see the gradual fading out of the normal reaction to electrolytes, and the assumption of the one peculiar to acid-modified protoplasm. This change in structural reaction always

accompanies the neutralization of the alkalinity of the culture. The complete reversal in the sign of the charge carried by the protoplasmic particles is apparently too fundamental a change to occur without killing the protoplasm except under the most perfect conditions. While much more work must be done on this point, we can assuredly say that the normal electrical charge carried by the protoplasm of *Paramœcium* bears a very close relation to certain chemical conditions of the environment, of which the alkalinity of the surrounding medium may be taken as one of the most important. These results are especially significant in the light of the behavior of paramœcia from various cultures toward different forms of stimuli, as we shall see when we discuss this subject.

III. *Reactions of the Protoplasm of Paramœcium to the Electrical Current.*

The above conclusions relating to the ultimate physical structure of protoplasm and the electrical conditions underlying it, are further borne out by a study of the effects produced on the structure of the protoplasm by the constant current. It has long been known that the constant current has a profound polar effect on the protoplasm of various protozoa.

Thus Kühne, Verworn[1] and others have shown that when a protozoan is exposed to the action of a weak current, a contraction occurs on the anodal side of the cell, and a relaxation on the cathodal side. This phenomenon was first described for *Actinosphærium*, a large heliozoan with many radiating pseudopodia. Very soon after exposure to the current the pseudopodia on the anodal side become contracted into irregular shapes, and are finally completely withdrawn into the cell, while those on the cathodal side remain fully extended. *Amœba* is still more sensitive to the current. The whole cell contracts on the anodal side while pseudopodia are rapidly thrown out toward the cathode so that the animal moves in this direction. The same general changes have been observed in *Paramœcium*[2] and many other protozoa. If the organisms are exposed to the current for a

[1] Verworn, *Arch. f. d. ges. Physiol.*, 1889, XLV., p. 1.
[2] Pearl, *Amer. Jour. of Physiol.*, 1900, IV., p. 96.

longer time, more pronounced structural changes ensue. The contraction on the anodal side continues until the protoplasm at this point can be seen to disintegrate, forming dense granular masses, while on the opposite side the protoplasm becomes even more clear and transparent than it was at first, frequently flowing out over this portion of the cell in irregular liquid masses. The observations have been repeated in a large number of forms so that the facts of definite polar modifications in the structure of the protoplasm during the passage of a weak constant current seem to be very well established. At the same time it has been shown in many forms that there is a movement of the protoplasmic particles away from the cathodal side where liquefaction of the protoplasm is taking place toward the anodal region of the cell where the disintegrating or "etching" effects are shown.[1]

I have repeated these experiments and have furthermore shown that when paramœcia are isolated in a weak gelatine solution or held in a fine mesh-work of cotton during the passage of the current so that they are unable to move freely from pole to pole, a slightly different structural reaction to the current is obtained after the current has been passed for from three to five hours. In this case all those paramœcia which are held in the region of the anode are modified as was the anodal side of the cell in the preceding experiment, *i. e.*, the whole cell contracts into a dense, opaque mass of protoplasm. Likewise the paramœcia held about the cathode are modified like the cathodal side of the cell in the former experiment, *i. e.*, the cell contents are liquefied and there is formed a large transparent cell of fluid protoplasm which ultimately bursts because of the increased pressure on the cell wall. Thus we have the same structural changes occurring either in the whole cell immediately about the anode, or in the anodal side of any of the cells exposed to the current; opposite changes occurring in the cells about the cathode or on the cathodal side of all the cells in the preparation.

A microscopical examination shows that these changes are

[1] Wallengren (*Zeitschr. f. allg. Physiol.*, 1903, III., p. 22) states that in the Rhizopoda only can the movement of the protoplasmic particles toward the anode be demonstrated. In the Infusoria the constant current does not affect the normal streaming of the protoplasm.

identical with the structural changes produced by the action of electrolytes upon the protoplasm. About the anode and on the anodal side of the cells the protoplasm is coagulated as by the use of cathions in weak solutions; about the cathode and on the cathodal side of the cells, the protoplasm is liquefied as by the use of anions in weak solutions. Not only are these structural effects the same, but they can be shown to be produced by the same means in each case, *i. e.*, by electrically charged ions, which are present in very dilute solutions of electrolytes, as we have shown, and which serve to carry the current from pole to pole when it is passed through such weak salt solution as the culture media of paramœcia which were used in the experiment.

It was formerly supposed that these polar effects were due to the formation of acids on the anodal side of the cell and alkalis on the cathodal side, but we have no satisfactory evidence for such internal polar changes. During the passage of the current, the anions prevail about the cathode, and are constantly diffusing toward the anode. During this passage they are continually impinging on the cathodal side of the paramœcia, hence a liquefaction of the protoplasm takes place at these places. Cathions prevail about the anode, and continually impinge on the anodal side of the cells in their diffusion toward the cathode so that coagulation occurs at these points. In either case the structural changes are produced by virtue of the electrical charge which the ions carry, and not by any specific chemical effect of the ions or molecules. We thus see that the chemical and electrical means of modifying the protoplasmic structure are identical.

It will be remembered that these changes in the structure of the protoplasm produced by solutions of electrolytes or the electrical current are the same as those which are brought about in organic colloids by the same means. The alkali-modified colloids in Hardy's experiments were coagulated by cathions in solution or at the anode during the passage of the current. They were liquefied by anions or at the cathodes. It is interesting to note also that in the protozoa a movement of the protoplasmic particles within the cell toward the anode has been observed, which corresponds exactly with the movement in the same direction among the colloidal particles of an alkali-modified colloid. Like

the colloidal particles, they always move toward the point at which coagulation occurs.

All these experiments seem to show that the structural changes produced in protoplasm by thermal, osmotic, chemical or electrical changes are the same, because all of these variations in the external conditions act upon protoplasm only by altering the physical state of its solid elements. Thus in the case of *Paramœcium*, at least, the structure of the protoplasm is seen to be not fixed and uniform, but to depend directly on certain external conditions and to vary with their variations. The best expression of this behavior of protoplasm is found in the laws of the reaction of colloidal solutions to external conditions.

Effect of these Structural Modifications on the Vital Properties of the Protoplasm.

Having determined the changes produced in the structure of the protoplasm by various chemical agencies, it remained to ascertain how these structural changes modify the vital properties of protoplasm. How far may a particular state of protoplasmic activity be correlated with a given physical condition of protoplasmic structure? Or does the reaction of an organism as a whole to an external stimulus depend in any measure upon the effect that stimulus may have on the structure of the protoplasm?

I. Growth and Cell Division.

The rate of cell division may be taken as the best indication of the general protoplasmic activity among the Protozoa, after the method adopted by Calkins[1] in his work on the "Life Cycle of Paramœcium." A quickened rate of cell division means an increase in the metabolic activities of the cell. A condition of slow metabolism is indicated by the cessation of cell division and the transformation of the motile cell into a spore or cyst or other resting stage. It has been already shown, in a paper[2] on the reactions of various protozoa to variations in the temperature, that precisely those temperature conditions, which liquefy the protoplasm, stimulate cell division, and those temperatures which coagulate

[1] Calkins, *Arch. f. Entwickelungsmech.*, 1902, XV., p. 139.
[2] Greeley, *Amer. Jour. Physiol.*, 1902, VI., p. 122.

the protoplasm inhibit it. Thus the rate of cell division increases steadily with a slight elevation of temperature above the normal until the critical coagulating point is reached. A lowering of the temperature progressively decreases the rate of cell division, until the point is reached at which it ceases altogether, and the protoplasm goes into a resting condition.

The same relation between the rate of cell division and the physical state of the protoplasm is found to hold good also as a result of the reactions of the protoplasm of *Paramœcium* to solutions of electrolytes.

With paramœcia from alkaline cultures, anions or liquefying agents stimulate cell division, cathions and coagulating agents inhibit it. Thus I have frequently observed in my experiments that when the liquefying solution is too weak seriously to modify the structure of the protoplasm, it will, however, greatly increase the motility of the protoplasm and the rate of cell division. Since the size of the paramœcia remains uniform, it follows that this must indicate also increased growth and general metabolic activity. In the coagulating solutions, on the contrary, there are produced spherical resting cells that greatly resemble spores. This antagonism between these two classes of solutions is still more clearly shown by the fact that the anions greatly accelerate the germination of spores, or the passage from a resting into a motile condition. In these solutions the spherical form of the spore is soon lost through a neutralization of the surface tension. This has been shown in the case of some of the monads and fresh-water algæ.

R. S. Lillie[1] has shown that a decrease in the surface tension must accompany cell division, and that this is accomplished in the case of certain marine animals by the electrolytes present in the sea water, for if these electrolytes be withdrawn, cell division not only stops, but a partial fusion of the already formed blastomeres occurs. It appears that the surface tension relations are very important in all these protoplasmic reactions to external conditions. Protoplasmic movement, cell division and growth all occur in opposition to the surface tension force.[2] Consequently any external condition which neutralizes the surface ten-

[1] Lillie, BIOL. BULL., 1903, IV., p. 164.

[2] See Spaulding, BIOL. BULL., 1904, VI., p. 97.

sion accelerates these expressions of the general activity of the protoplasm. These conditions are those which bring about a liquefaction of the protoplasm, so that the protoplasmic activity is seen to vary directly with the amount of water the protoplasm contains. Conversely the assumption of a quiescent spherical resting stage in various protozoa is the result of an increase in surface tension, and is formed by those conditions which cause a coagulation of the protoplasm and a loss of water. Thus a slight increase in the temperature is seen to have the same effect on these simple protoplasmic properties of *Paramœcium* as anions ; a lowering of the temperature acts like cathions ; since each set of conditions produces the same structural effects.

II. *The Tropisms.*

We have at the present time an enormous amount of information concerning the reactions of organisms to external stimuli, but we know almost nothing of the physical or chemical effects of these attractive or repellent agents on the protoplasm of the organism, and consequently we are not able to offer any satisfactory explanation of the mechanism of the tropic response. In the following experiments I have studied the reactions of paramœcia to thermal, electrical and chemical stimuli, and have attempted to show that the reaction of *Paramœcium* to each stimulus depends on certain structural changes in the protoplasm, which are a result of the stimulating action.

1. *Thermotaxis.* — The reactions of paramœcia to variations in temperature are exceedingly definite. It has been stated by many observers that they are positive to temperatures between approximately 23° and 27° C., and are negative to all others. This reaction is beautifully demonstrated by placing the paramœcia in a long, narrow dish which is heated at one end and cooled at the other. It has been already shown that those temperatures to which *Paramœcium* is positive constitute exactly those thermal conditions which bring about a liquefaction of the protoplasm and a reduction in the surface tension. Those temperatures to which *Paramœcium* are negative, coagulate the protoplasm. We thus see that, in the case of thermotaxis, attraction is accompanied by a liquefaction of the protoplasm, repulsion by coagulation. The

extreme delicacy of the adjustment between the physical structure of the protoplasm and the external conditions has hardly been recognized. For example, the smallest perceptible elevation of temperature above the normal results in a decided increase in the fluidity of the protoplasm, and it is probable that this structural change explains the extreme sensitiveness of the paramœcia, judged by their thermotropic reactions, to these same variations in the temperature.

2. *Galvanotaxis.* — It has been well known that paramœcia normally react to the electrical current in a vigorous and definite manner by orienting themselves with their anterior end toward the cathode, and swimming rapidly in this direction, so that eventually a dense gathering of the organisms occurs about the negative electrode. In other words they collect at that point in the electrical field where the conditions are such as to induce a liquefaction of the protoplasm. After a weak current has been passed through the preparation for from thirty minutes to one hour, it will be seen that the dense gathering at the cathode begins to break up and a reverse movement toward the anode sets in. The number of paramœcia that exhibit this reverse movement varies with the conditions of the organisms at the time of the experiments, as will appear later; but with paramœcia from alkaline cultures only a small proportion of the entire number will be seen to swim toward the anode at any one time. At first the paramœcia swim only a short distance toward the anode and then immediately dart back to the cathode, but the length of the reverse reaction increases until a few reach the anodal end of the dish. Having arrived at the anode, they immediately swim back to the cathode again, and no gathering occurs at the anode except in rare cases after the current has been passed for about two hours. The paramœcia normally keep the anterior end pointed always toward the cathode, so that they swim backwards toward the anode. But this is not always the case.

After a large number of experiments with paramœcia under various conditions, I find that the relations between this initial and secondary reaction to the current may be greatly modified. With paramœcia from alkaline cultures, the secondary reaction begins only after the current has been passed for thirty minutes

or more and is at first of an exceedingly transitory nature. But with paramœcia that have been reared in a culture that has been made slightly acid by the fermentation of starch, and hence are in an acid-modified condition as before described, the secondary reaction may begin as soon as the paramœcia reach the cathode. In these cases no gathering occurs at the cathode but each *Paramœcium* immediately reverses the stroke of the cilia and swims back to the anode. The process is repeated at that point, so that we have for a time no collection at either pole but a continuous line of paramœcia swimming in either direction until eventually they come to rest in about equal numbers at each end of the preparation. Under the most favorable acid conditions a number of paramœcia, varying from one to fifty per cent. of the whole number, exhibit an initial reaction toward the anode and a secondary reaction toward the cathode, while the remainder react in the manner described above.

That this immediate reversal of the normal reaction and the initial response toward the anode are due to the acidity of the culture medium may be shown by the following experiments. If 5 c.c., of a neutral [1] culture of paramœcia be isolated and tested to the current, it will be found that they all exhibit the characteristic response toward the cathode and form a dense gathering at that point. If now, however, from two to four drops of an $m/10$ solution of hydrochloric or other acid be added to the culture, and, after standing for thirty minutes, the reaction to the current be again tested, it will be found that either an initial or an immediate secondary reaction toward the anode has set in. Also the addition of a small amount of acid to an already acid culture invariably strengthens the anodal response of paramœcia. Likewise in every case in which I have tried it, the neutralization of the acid with NaOH, or the addition of the solution of a salt with a trivalent anion like Na_3PO_4 or $Na_3C_6H_5O_7$ entirely destroys both the initial and the secondary response toward the anode, and leaves only the characteristic gathering at the cathode.[2]

[1] It is necessary to use paramœcia from an approximately neutral culture for this experiment. The normal reaction toward the cathode is too firmly fixed in paramœcia from a strongly alkaline culture to be reversed.

[2] More striking results have been obtained with *Volvox*. After an exposure of half an hour or more to a slightly acid medium, practically every organism completely reverses its response, so that a dense gathering is formed about the anode.

In several instances, after a prolonged exposure to a medium which had been acidulated by the addition of hydrochloric or acetic acid, the paramœcia exhibit still another form of reaction. In these cases the paramœcia orient themselves transversely or slightly obliquely to the direction of the current, and swim very slowly from side to side of the preparation. At the same time, however, the organisms appear to drift passively toward the anode. I observed this form of reaction only about half a dozen times, but on each occasion the reaction was exceedingly well marked. A reversal of the current caused an instant reversal, of both the direction of the swimming and the passive drifting of the organisms. Notwithstanding the peculiar orientation of the paramœcia, they all tended to form a gathering about the anode under these acid conditions.

It has been observed by Loeb, Jennings and others that the addition of other substances, like NaCl, to the solution containing the paramœcia will cause a reverse movement toward the anode. I investigated this question and found that the addition of almost any salt in sufficient quantities to extract water from the protoplasm will cause a more or less complete reversal. A large number of salts, of both positive and negative electrical conditions, were used, and the effect was seen to be purely osmotic in character, except in the case of the salts with trivalent anions or cathions. The former, as the phosphates and citrates, act like the hydrates in very weak solutions and completely destroy all traces of a response toward the anode. The latter, as Al_2Cl_6, produces an almost instant coagulation of the protoplasm and hence bring about the same effect as the extraction of water osmotically. It has been already stated that a lowering of the temperature coagulates the protoplasm, and it is interesting to note, that a partial reversion of the electrical response may be produced by this means also. After the paramœcia have been exposed to a temperature of 2° to 3° C., for one hour or more, the normal response is entirely lost, and a slight movement toward the anode can be detected.

Our ignorance of the precise nature of the ciliary response is too great to allow even an attempt at an explanation of the mechanism of this response to the electrical current. We will

first have to obtain a satisfactory explanation of the rhythmical contraction of the ectosarc which controls the cilia. It is certain that the surface tension relations of the muscular elements play an important rôle in this process. In *Amœba* the problem is very simple. The protoplasm contracts on the anodal side because of the neutralization of the charge carried by the protoplasmic particles and consequent increase in surface tension. Pseudopodia are thrown out on the cathodal side, and movement occurs in this direction because of the decrease in surface tension at this point. But for paramœcia it suffices at present to show that the sense of the response is not a fixed attribute of the organism which has been acquired by natural selection, but that it is a purely physical response to an external stimulus, and varies directly with the conditions under which it occurs. The ultimate determining factor of the response to the electrical current must be the electrical conditions of the protoplasm itself. In paramœcia from a normal alkaline culture the protoplasmic particles appear to be negatively charged. These paramœcia collect about the cathode where liquefaction of the protoplasm occurs. But with precisely the same conditions, under which liquefaction is produced not by anions but by cathions, *i. e.*, an acid culture medium, we find that the characteristic gathering about the cathode does not occur, but the paramœcia tend to move toward the anode where the protoplasm would now become liquefied. The only explanation of this phenomenon that presents itself is the assumption, that in an acid medium the protoplasmic particles become positively charged in a portion of the organisms (for the reaction is never completely reversed). The partial reversal of the reaction by osmotic means is also due to an alteration in the electrical conditions of the protoplasm, as has been shown.

Hardy's acid and alkali-modified colloids reacted also in an opposite manner to the electrical current because in the acid solution the particles were positively, and in the alkaline solution negatively charged; and while the explanation is not so simple in the case of *Paramœcium*, because movement is effected by a complex motor apparatus, still the sense of the reaction must be ultimately due to the same cause in both cases, *i. e.*, the charge carried by the colloidal or protoplasmic particles. Moreover,

Hardy observed the same secondary reversed movement of the colloidal particles as has been observed in the reaction of *Paramœcium*. All these facts make very evident the similarity which exists between the electrical conditions in the two solutions. Lillie[1] has observed a similar relation between the response to the electric current and the chemical condition of the protoplasm whether acid or alkaline, in his experiments upon nuclear and cytoplasmic structures cited at the beginning of the paper. He shows, that, when exposed to the electric current, nuclear structures, which contain a large amount of nucleic acid, move toward the anode, while cells very rich in cytoplasm, which is basic in reaction, move toward the cathode.

3. *Chemotaxis.* — A large number of important contributions have been made during the last few years to our knowledge of the reaction of protozoa to chemical stimuli. Most notable have been the remarkable series of investigations carried on by Jennings, who has given us not only a complete account of the sense of the reaction of many protozoa to a wide range of chemical substances, but also an accurate description of the method of the reaction in each case. My own results agree with those of Jennings in all essential points. I confirm his account of the "motor reaction" of *Paramœcium* when under a chemical stimulus. It was not my purpose to repeat any of Jennings' experiments, but only to ascertain the chemotropic reactions of *Paramœcium* under various conditions, to see if they could be modified by external influences as was the galvanotropic response. The only respect in which my conclusions depart from Jennings' is that my experiments seem to show that the chemotropic reactions of *Paramœcium* which he describes are not of universal occurrence, but limited to paramœcia which have been reared under definite chemical surroundings. In other words, the sense of the chemotropic reaction, like the galvanotropic, depends upon certain chemical conditions of the environment.

Jennings found that paramœcia were in general positive to weak acids (*i. e.*, formed a gathering within the drop) and negative to weak alkalis. He also observed that they reacted in a constant manner to a large number of salts. In my own experi-

[1] Lillie, *loc. cit.*

ments, I find, that only those paramœcia which have been reared in a slightly acid culture medium are positive to acids, and that paramœcia from clear alkaline cultures are negative to acids and positive to alkalis. It appears also that salts with trivalent cathions act like acids, and salts with trivalent anions act like alkalis. No definite conclusions could be drawn from the action of the univalent and bivalent salt solutions. For the purpose of the experiments, we are most vitally concerned with the reaction of those solutions which carry the heaviest positive or negative charges of electricity.

The greatest caution is needed in determining the acidity or alkalinity of the culture medium. A carefully prepared litmus solution is the best indicator. Phenol-thalein may also be used for the detection of small amounts of alkalis. Paramœcia freshly reared in a culture whose alkalinity has been determined in this way invariably react to the solutions used as follows: To $m/200$ HCl, HNO_3, H_2SO_4, and acetic acid; $m/800$ Al_2Cl_6 and Fe_2Cl_6, they are negative. To $m/200$ NaOH, KOH, $Ba(OH)_2$ and $Sr(OH)_2$; $m/480$ $Na_3C_6H_5O_7$ and Na_3PO_4, they are positive. The positive reaction is shown by the organism swimming into the drop, and then giving the motor response, described by Jennings, when they come in contact with the outside water, so that a gathering is formed within the drop. The solutions to which the paramœcia are negative provoke the motor response when the organisms first come in contact with the outer edge of the drop, with the result that the solution is left empty. In some cases the paramœcia appear to be entirely indifferent to a solution, and swim in and out in an undisturbed manner. Such a reaction will also be classed as negative.

If the paramœcia from the same alkaline culture be tested from day to day as the alkalinity is being gradually neutralized by the formation of acid during the fermentation of bread, or by the addition of free acid to the culture, it will be seen that the response to these solutions slowly changes, until finally the chemotropic reaction is completely reversed, and now in the acid medium the paramœcia are positive to acids and salts with a trivalent cathion, and negative to alkalis and salts with a trivalent anion. Likewise, if the culture be again made gradually alka-

line, the first form of reaction, characteristic of alkaline cultures, returns, so that it becomes evident that the sense of the chemotropic reaction depends directly on certain chemical conditions of the surrounding medium. The transformation from the first form of reaction to the second, and *vice versa*, is a very gradual one, so that it is not immediately effected by the chemical change in the surrounding medium, and a considerable time may elapse between the neutralization of the alkalinity of the culture, for example, and the loss of the positive response to alkalis ; but eventually the reaction occurs as has been described. Thus paramœcia are seen to seek out those chemical conditions which bring about a liquefaction of the protoplasm. The sense of this response also is apparently determined by the electrical condition of the protoplasm.

An interpretation of the mechanism of this response to electrolytes is as impossible as it was in the case of the reaction to the electric current. But, since in each case the paramœcia collect under the same electrical conditions, both responses must be ultimately due to the same reaction of the contractile layer of the protoplasm to electrically charged ions, and this reaction must consist largely in the effect which the electrically charged ions have upon the surface tension of the contractile elements of the protoplasm. Experiments upon *Amœba* bear out this hypothesis. Cathions always produce a contraction of the protoplasm, while anions produce a relaxation or the extension of pseudopodia, because the former increase the surface tension of the protoplasm while the latter neutralize it. Thus *Amœba*, like *Paramœcium*, is positive to predominately negative solutions, but in the one case the response is accomplished by the immediate effect which the anions have upon the surface tension of the protoplasm, while in the other case it is brought about through the agency of a complex motor apparatus.

Conclusions.

We see that precisely those chemical changes in the surrounding medium, which modify the structural reactions of the protoplasm of *Paramœcium* to solutions of electrolytes, modify also the reactions of the organism to electrical and chemical stimuli.

The protoplasm of paramœcia from an alkaline culture is liquefied by temperatures between 23° C. and 30° C., by anions, and at the cathode during the passage of the constant current. The paramœcia also react positively to all these chemical and physical conditions. The protoplasm of the same paramœcia is coagulated by temperatures below 20° C. and above 30° C., by cathions, and at the anode during the passage of the current. The organisms are negative to all these conditions. The structural changes produced by electrolytes are partially reversed in paramœcia from a slightly acid culture, and the reactions of the organisms are also partially reversed to the electric current, completely so to solutions of electrolytes. In every case the reaction of a *Paramœcium* to an external stimulus leads it to remain under those conditions which liquefy the protoplasm. Attraction is accompanied by liquefaction, repulsion by coagulation. As far as the physical structure of the protoplasm is concerned, the conclusion from these facts seems to be that the protoplasmic particles are physically identical with colloidal particles. Hence the protoplasm of *Paramœcium* is essentially a colloidal solution whose particles carry a definite charge of electricity. The sign of this charge appears to depend on certain external chemical conditions, of which the alkalinity of the surrounding medium may be taken as one of the most important. The sign of this charge is seen to determine not only the structural modifications of the protoplasm, but also the reactions of the paramœcia to chemical and electrical stimuli. This conception of the physical structure of protoplasm is also used to explain the effect of external conditions on the processes of cell division, growth and movement through the operation of the laws of surface tension.

Zoölogical Laboratory,
Washington University.
February 10, 1904.

EXPERIMENTAL STUDIES ON THE DEVELOPMENT OF ORGANS IN THE EMBRYO OF THE FOWL (GALLUS DOMESTICUS).

FRANK R. LILLIE.

II. The Development of Defective Embryos, and the Power of Regeneration.[1]

Born has shown that young embryos of the frog possess immense power of healing smoothly cut wounds, and that the vitality of even small isolated parts is remarkable ; pieces of the head or of the tail, for instance, may continue to grow and develop in spite of the complete absence of the heart, blood and blood vessels, and nervous system, for a period of about three weeks, or until the yolk contained in the cells is fully consumed. "The development of each organ progresses as far as the cut surface as well as in the normal larva, no matter what the position of the cut surface may be ; the absence of the heart or the brain does not affect the subsequent processes of growth and differentiation in any marked way."

These conclusions are of great importance for the physiology of development. So far as I know, similar experiments have not been performed on the embryos of *Amniota*, and the following account may serve to fill up the gap in part. Striking differences appear between the embryo of the fowl and of the frog in regard to the vitality of defective embryos, or of parts of embryos : The chick embryo dies very rapidly after destruction of the heart, for the embryonic tissues are dependent to an extreme degree upon the circulation ; it therefore follows that small parts of an embryo cannot undergo differentiation as in the frog. On the other hand, the growth of the extra-embryonic blastoderm is independent of blood supply, and may continue in eggs in which there is no embryo owing to destruction of the heart, until nearly the entire yolk is covered. This difference between the embryo of the chick and its own extra-embryonic blastoderm or the embryo of

[1] The first part appeared in Biol. Bull., 1903, Vol. V., No. 2 : "Experiments on the Amnion and the Production of Anamniote Embryos."

the frog, may be explained by the simple consideration that the cells of the embryonic tissues proper in the chick are devoid of yolk or other nutriment, and hence are dependent for their subsequent growth and differentiation upon the circulation ; whereas the cells of the frog embryo are loaded with food in the form of yolk ; and the extra-embryonic blastoderm of the chick is a digestive organ lying on an immense reservoir of food.

The necessity of circulation for all normal development of the chick embryo beyond the stage of about 33 hours (12–14 somites) at once limits the range of defective embryos capable of development to those possessing a heart and vitelline circulation. Another limitation arises from the extreme sensitiveness of the embryo to removal of parts of the brain ; although I have made over seventy experiments on the brain, none of the embryos, in which the injury extended back of the optic stalks, has developed for more than about forty-eight hours after the operation. (The operations on the head form a class in themselves and will be discussed in a separate paper.) The reason for the large number of fatalities in operations in this region is probably not due to any trophic function of the nervous system, at least in stages younger than 72 hours, but either to direct injury to the anterior end of the heart, or to malformations of the amnion consequent on the operation. On the other hand, embryos may survive the destruction of a considerable portion of the posterior end, and develop normally for several days as least. All the defective embryos to be described resulted from operations of this kind, performed, with one exception, on embryos in which the tail-bud is just forming after 50–60 hours' incubation.

A larger or smaller part of the posterior end was destroyed by cauterization. The embryos might bear complete destruction of the posterior end up to the vitelline arteries, provided these were not injured, without any apparent detriment or hindrance to the development of the uninjured parts. If the vitelline arteries were destroyed, the embryo never survived. Fig. 1 gives a view of an embryo of about the age of those used for operations. In this case 29 somites are formed ; the number was certainly not over 30 at the hour of operation in any case. The somites are continued backward by the undivided segmental plate represent-

ing a considerable number of potential somites. As will be shown later, somites 26 to 32 are the ones that normally form the musculature of the leg. Thus the undivided mesoblast at this stage includes a large part of the trunk. The vitelline arteries at this time leave the embryo opposite to the twenty-first or twenty-second somites; this position appears to be very constant. Thus the theoretical limit of the experiments is from the hind end to about the twenty-second somite, because

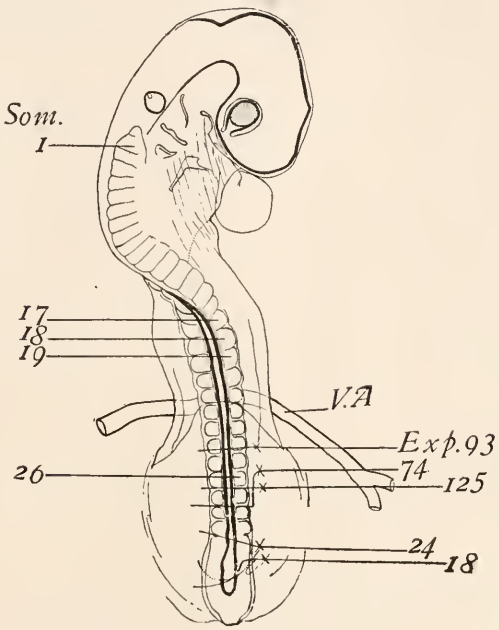


FIG. 1. Camera drawing of chick embryo with 29 somites; operation diagram. For explanation see text.

the embryo cannot live if the vitelline arteries are destroyed. The rudiment of the allantois lies beneath the unsegmented mesoblast beginning, approximately, a short distance back of the thirtieth somite.

Fig. 1 serves at the same time as a diagram of the operations. At the time of each operation a sketch was made of the embryo, and the part destroyed was indicated by shading the posterior end correspondingly. This sketch, naturally, did not show somites, so the present operation diagram is constructed from data deter-

mined after the operation. It agrees, however, fairly well with the original operation diagrams which were simply rough estimates of the amount destroyed by the operation. It should be distinctly understood that the figure is a result of study of the anatomy of the defective embryos. In further explanation it should be added, that the numbers to the right are the numbers of the experiments ; those to the left of the somites. The lines leading to the numbers indicating the experiments are drawn across the body to indicate, that, in the experiment in question, all back of the line was destroyed ; the cross on each line marks the junction of reference and operation lines.

A word concerning the enumeration of the somites ; the somite numbered 1 possesses a short anterior process, that is probably an independent somite. It is, however, so inconspicuous in many embryos that it seemed better for the present purpose not to enumerate it. Somites 17, 18 and 19 are especially marked because they are the wing-somites, *i. e.*, the somites that will form the major portion of the bone and muscle of the wing. The twenty-sixth is the first leg somite. By this reckoning, there are three cephalic somites, or, reckoning in the incomplete one, four.

Referring to Fig. 1 again, it will be seen that the organs destroyed in such operations are : (1) The hind end of the neural tube ; (2) the hind end of the notochord ; (3) the mesoblastic segmental plate and often certain of the posterior mesoblastic somites ; (4) the hind gut including the rudiment of the allantois ; (5) the hind end of the Wolffian body and ducts.

I may say at once that no true regeneration of these structures takes place. (For discussion of this, see p. 50.) Therefore the problems of interest became narrowed down to the differentiation of the uninjured parts, and my attention has been particularly drawn to the behavior of the mesoblastic somites. In the somites we have an originally homonymous series of structures, the segmentation of which becomes strikingly heteronymous as development proceeds, some entering into the head, others the neck, others the wing, the thorax, abdomen, leg and tail. Definite somites, *i. e.*, somites in a definite numerical position in the series, form the skeleton and muscles of each of these regions. It would

be interesting to determine whether or not somites had the same rôle in defective as in normal embryos.

The numerical value of the somites in the chick seems to be normally as follows :

96-hour Chick.		Eight-day Chick.	
1, 2, 3,	Cephalic, hypoglossus region.[1]		
4 to 16,	Prebrachial, trunk.	1 to 13,	prebrachial.
17, 18, 19,	Brachial.	14, 15, 16,	brachial.
20 to 25,	Between wing and leg.	17 to 22,	between wing and leg.
26 to 32,	Leg somites.	23 to 29,	leg.
33 to 35,	Region of cloaca,	30 to 32,	region of cloaca.
36 to 42,	Caudal somites.	33 to 38,	caudal.

The count in the eight-day chick is really an enumeration of nerves. For the four-day chick the exact number of the leg somites was determined by comparison with the enumeration of nerves of the eight-day chick. The wing somites may readily be distinguished at four days, in entire mounts, by their size and by the relatively large amount of mesenchyme formed opposite them. In embryos of more than four days of age the enumeration by nerves is much the easier ; and, as the brachial nerves (14, 15, 16) are very much larger than their neighbors (Figs. 3 and 5, *B.P.*), it is simplest in determining the place of a postbrachial somite or nerve to count only the somites back of the last brachial nerve. Thus the postbrachial somites 1–6 are between arm and leg ; postbrachial 7–13 are leg-somites, etc.

Experiment 125.

In this experiment a very considerable part of the hind-end was destroyed at the time of appearance of the tail-bud (see operation-diagram), and the embryo was allowed to develop for about four days more (91 hours). The egg was then reopened. The vascular area covered at least three fourths of the yolk. There was no allantois. The embryo lay in large part beneath the blastoderm, but a large aperture in the latter towards the hind-end of the embryo was filled by the amnion through the transparent walls of which the embryo could be distinctly seen. A large part of the blastoderm was removed with the embryo and examination of the under surface was then made in salt solu-

[1] In this enumeration I have omitted again the incomplete anterior cephalic somite.

tion. The amnion was very well distended, and attached round the margins of the somatopleure at the hind-end. Behind this

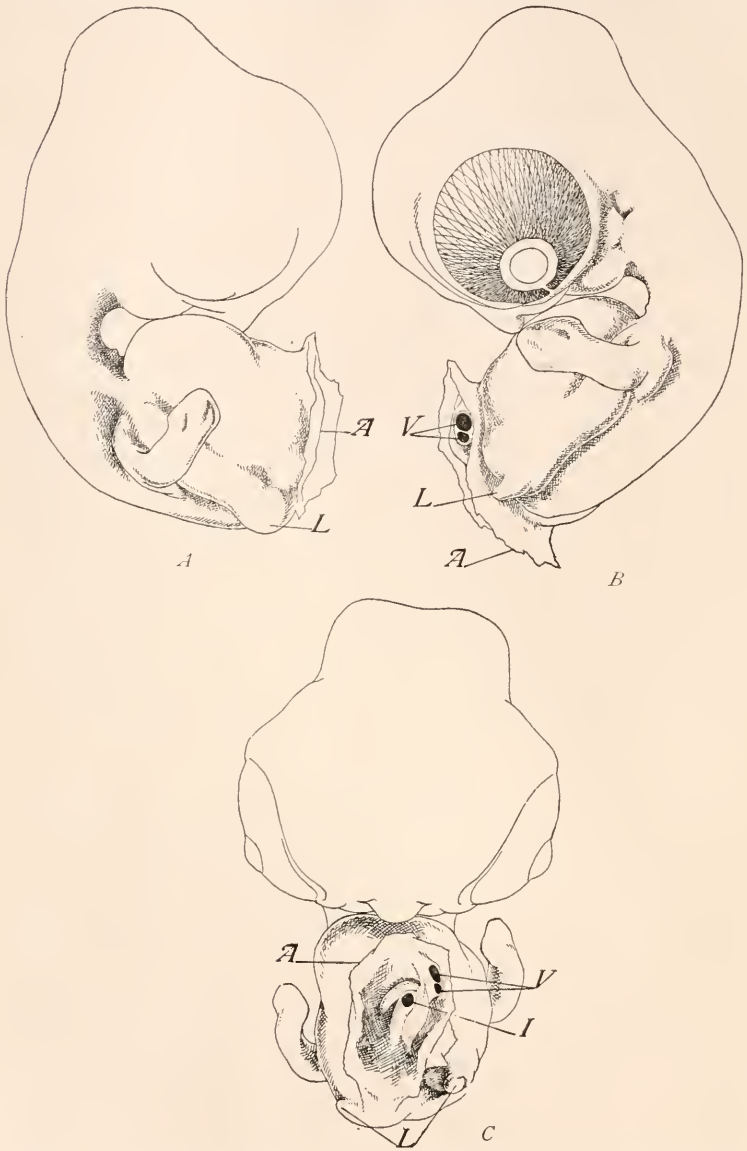


FIG. 2. Three views of a defective embryo (experiment 125) with membranes removed; total age about six days. *A*, amnion; *I*, ectopic intestine; *V*, vitelline artery and vein; *L*, rudiment of hind limbs.

the embryo was naked and there was slight ectopia of the intestine (Fig. 2, *C*).

Figures 2, *A*, *B* and *C* show this embryo from the two sides and from behind. The last figure shows especially well the attachment of the amnion and the ectopia of the intestine external to this. The hind-end of the embryo back of the vitelline vessels is wanting. On the other hand, all parts in front of this are normally developed.

On each side, at the hind-end of the Wolffian ridge, rudiments of the hind limbs are present as prominent conical stumps. From this it would appear at first sight that there has been some regeneration. However the question can be decided only by ascertaining the numerical value of the segments concerned in the formation of these stumps. The embryo was cut into sagittal sections for this purpose.

The enumeration of the post-brachial nerves is the same on both sides, seven pairs being present. The stumps are innervated only by the seventh on each side (Fig. 3). In the normal chick, the nerves innervating the leg are the seventh to the thirteenth postbrachial. Thus it would appear that only one leg somite on each side was uninjured by the operation, and this is the only one that has contributed to the formation of the rudimentary leg. None of the six somites between arm and leg has undergone any alteration of its normal numerical value. This result must therefore be interpreted in the sense of normal self-differentiation of the somites concerned.

Structure of the Leg-rudiments. — But, even though the deficiency of leg somites has not stimulated their immediate neighbors in front to any act of supererogation, it might be that the only leg somite remaining has produced more than its wont. In this connection the structure of the stumps is of interest: Owing to their positions, the left one is cut longitudinally and the right one transversely in the sagittal series. The structure is the same on the two sides (see Fig. 3); there is a single curved rod of precartilage extending out nearly to the tip of the limb; this is surrounded by a mass of dense mesenchyma, evidently premuscle tissue, and externally are the elements of the skin. Now, in the normal limb of corresponding age, the skeleton of the thigh,

crus and pes are separate chondrifications. The crus and pes are thus certainly not represented in the rudimentary hind-limb of this embryo.

In the normal chick the seventh postbrachial nerve innervates only some of the proximal muscles of the leg, those extending from the pelvis to the femur. On the principle that the muscular distribution of the nerve is confined to the derivatives of the corresponding somite, it follows that the seventh postbrachial somite contributes to the formation of only the upper segment of the leg.

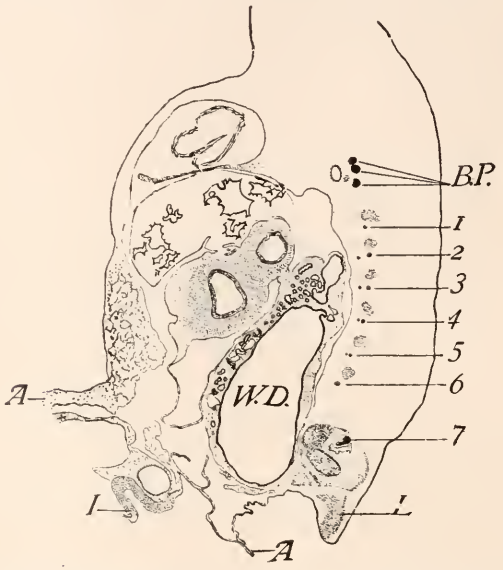


FIG. 3. Sagittal section of embryo shown in Fig. 2, cut considerably to the left of the middle line. *A*, amnion; *B.P.*, brachial plexus (nerves of wing); *I*, posterior end of intestine; *W.D.*, Wolffian duct; 1–7 first to seventh postbrachial nerves.

The absence of the crus and pes is therefore what might be expected if the principle of self-differentiation were rigidly adhered to. We have seen that, as a matter of fact, these are absent in the rudimentary hind limbs of this embryo, so we may conclude, at least, that there is no evidence that the seventh postbrachial somite has produced anything beyond the normal as a result of the absence of the following somites of the leg.

Alimentary Tract. — Practically all of the splanchnopleure of the embryo posterior to the vitelline arteries was destroyed by

the operation. In the resulting embryo, the trachea is a long tube, the lungs are budding out, the œsophagus is well formed, and the stomach is in two divisions, in the anterior of which the glands have begun to form, and the intestine is slightly coiled and opens on the hind surface, across which it is continued as a superficial strip ending in a free blind tag with a slight lumen (Figs. 2*C* and 3). The liver and pancreas are normally formed. In fact the parts present are apparently in the same condition that they would have been had the embryo been intact.

The allantois is entirely absent; and there is no evidence of any compensating growth of the intestine.

Nervous System. — The spinal cord ends bluntly, but the neural canal is prolonged backwards at its ventral angle into a short process which tapers into a bundle of neurites that may be followed a short distance, and then terminates abruptly. It would appear that the descending tracts in the cord have caused the prolongation, and have then pushed out into the adjacent tissues. A similar prolongation appears in all embryos defective at the hind-end (Fig. 3, *A*).

Excretory System. — The Wolffian ducts are much dilated, as there is no outlet for the secretion of the mesonephros (Fig. 3). No metanephric outgrowths have arisen from them, although in the normal embyro of this stage these are well developed. The metanephric region of the Wolffian ducts was of course destroyed by the operation; but, as the ducts are continuous structures, one would not anticipate that the capacity for producing metanephric outgrowths would be limited to a short stretch at the posterior end. Of course another explanation of their absence than that of limitation of potency to a short region of the Wolffian duct is possible, *i. e.*, that suitable predisposing external conditions for their formation are strictly limited (*e. g.*, that their development depends on a certain amount or kind of development of the allantois or cloaca).

Experiment 93.

In this experiment a very considerable portion of the hind-end was cauterized and removed (see operation diagram). Seventy-two hours later the egg was reopened and found living. The vascular area covered about three fifths of the yolk; the circulation was very active, and there was no allantois.

Fig. 4 is a view of the embryo in the amnion, drawn from the under side of the blastoderm. The vitelline blood vessels are attached to the center of the defective hind-end, the entire trunk back of these vessels being absent. The amnion is well distended; its line of attachment is indicated by the dotted line. Just behind this line of attachment is seen a little tag, the hind-end of the intestine. No trace of the hind-limbs is visible externally.

This embryo was cut into sagittal sections. In these there is no trace of the hind limbs, and no evidence that the posterior myotomes or sclerotomes are modified towards the formation of limbs. The embryo is not quite so far advanced in development



FIG. 4. Defective embryo (experiment 93) in the amnion; part of the folded under surface of the blastoderm is shown. *A*, amnion; *I*, ectopic intestine; *V*, vitelline arteries and veins.

as that of experiment 125, but it is certainly old enough to show the rudiments of the hind limbs or any part of them, were there any tendency towards their formation.

Study of the sections shows that there are only five pairs of post-brachial ganglia and nerves (Fig. 5), the fifth on both sides

being very small, and evidently partially destroyed by the operation. As the first leg-somite is the seventh post-brachial, the absence of rudiments of the hind limbs in this embryo is readily understood.

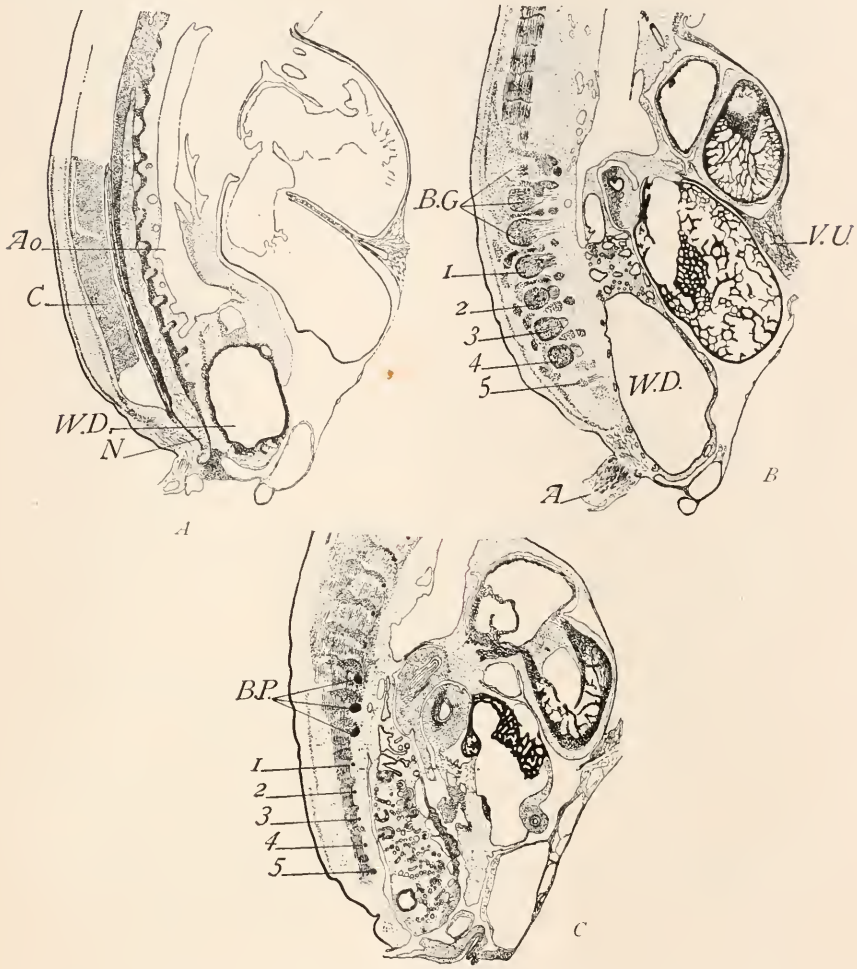


FIG. 5. Three sections from a sagittal series through the embryo shown in Fig. 4. *A*, amnion; *Ao.*, aorta; *B.G.*, ganglia of the nerves of the wing; *B.P.*, nerves of wing; *C*, cord; *N*, notochord; *V.U.*, umbilical vein; *W.D.*, Wolffian duct; 1–5, first to fifth postbrachial ganglia or nerves.

Thus there are two somites less in this embryo than in that of experiment 125. In the latter, which includes one leg somite, rudiments of the hind limbs are formed; in the present case no

such rudiments are formed, although the embryos are very much alike in every other way. It would be interesting to compare an embryo with six post-brachial somites, but I have none yet.

The central nervous system ends bluntly, except for a short prolongation of the ventral angle of the canal (Fig. 5, *A*). This condition, which is characteristic for all embryos defective at the hind-end, appears to be due to growth of fibers in the ventral motor zone, for a bunch of neurites extends back on each side of the ventral middle line nearly to the hind end of the notochord. The notochord extends backwards to the extreme posterior end, thus some distance further than the spinal cord. It thus appears, as in the other embryos of this class, to have undergone some regeneration, or at least growth, at the hind end.

The intestine opens at the posterior end, and is continued as a flat strip along the surface of the splanchnopleure. It is interesting to note that this strip has the same histological structure as the walls of the tube proper, showing that the histogenesis depends upon the character of the cells, and not upon such external factors as the form of the tube. The liver is normal. There is no trace of the allantois; but the stem of the umbilical veins may be seen, in a very rudimentary condition, running in the *septum transversum* to enter the anterior face of the liver (Fig. 5, *B*).

The *Wolffian bodies* are well developed, and the Wolffian duct much enlarged as in other anallantoic embryos (Fig. 5). Metanephric outgrowths are absent.

Summing up, we may say, that all the embryonic rudiments present have differentiated in the normal fashion. There has been no modification of the numerical value of the somites, no compensating growth, and no regeneration, unless we except the elongation of the notochord. Those embryonic organs, whose rudiment or ordinary locality was destroyed, are simply missing.

Experiment 74.

A large part of the hind end was destroyed at the stage of about 52 hours (see operation diagram). The egg was reopened about 68 hours after the operation. The vascular area covered fully half of the yolk; the heart-beat was vigorous.

Fig. 6 gives two views of the embryo. The hind end is entirely absent, and the opening is filled by the enlarged Wolffian ducts. The band of tissue between the two ducts is the hind end of the alimentary tract. The right side is seen to be less developed than the left. In the view from the opposite side, it can be seen that there is a rudiment of the hind-limb on the left side; but apparently none on the right.

This embryo was cut into transverse sections. Study of these shows that there are eight postbrachial ganglia on each side, though the eighth on the right side is smaller than on the left.

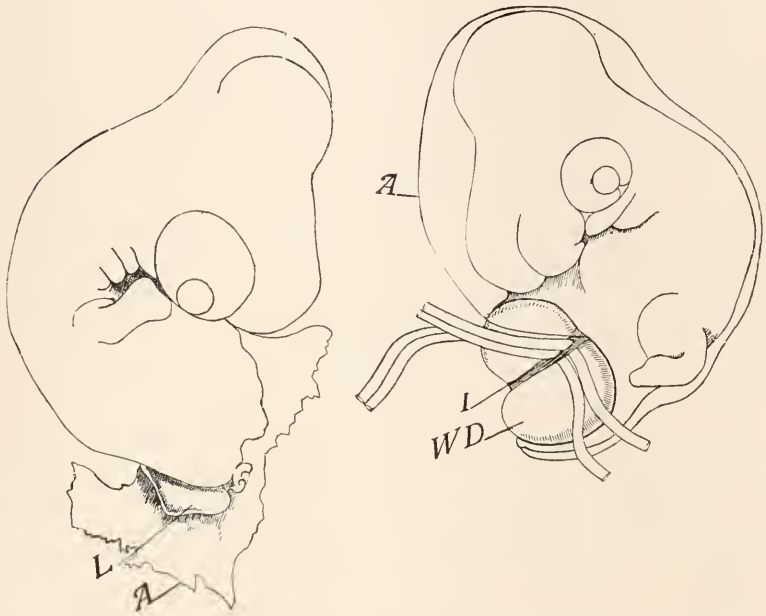


Fig. 6. Two views of a defective embryo (experiment 74). *A*, amnion; *I*, strip of intestinal epithelium; *L*, leg; *W.D.*, Wolffian duct. The vitelline blood vessels are somewhat abnormal.

On the left side the seventh and eighth postbrachial nerves enter the rudiment of the hind limb. On the right side there is no such rudiment, and the seventh and eighth nerves, which are quite large, end bluntly in the mesenchyme, almost as though cut off. The question arises why there is no limb rudiment on the right side? One can explain this by assuming that the operation destroyed the seventh and eighth postbrachial meso-

blastic somites on this side, but not the ganglia and cord of these somites (see Fig. 1). The form of the embryo indicates that this is true in part; but there is a small myotome in the eighth postbrachial somite of this side, and in the seventh the myotome is quite as well developed as on the other side. It would appear probable, then, that the most lateral portions of the seventh and eighth postbrachial mesoblastic somites were destroyed, together with the somatopleure lateral to this; either the rudiments of the hind limb were included in the destroyed parts, or their development was prevented by the scar-tissue. It is impossible to say whether or not the two somites concerned in the formation of the left leg have formed more than the normal.

The central nervous system terminates abruptly, except that the ventral portion of the canal is continued back as a narrow epithelial tube. The notochord extends some sections back of the termination of the neural tube, showing that it has been added to at its hind end since the operation.

The Wolffian ducts are immensely enlarged (see figures). There is no metanephric outgrowth. The allantois is entirely absent. The alimentary canal is normal back to the defective region and terminates in a band lying between the two Wolffian ducts (Fig. 6).

Experiment 18.

The tail-bud of the embryo was just formed at the time of the operation, and it was completely destroyed with a heated needle (see operation diagram). The injury did not extend so far forward as in the other cases mentioned and was asymmetrical (Fig. 1).

Fifty hours later the egg was reopened, and the embryo was found living, the heart beating actively; the vascular area covered about one third of the yolk.

Fig. 7 shows the embryo as it appeared from the under surface of the blastoderm. It will be observed that the amnion ceases with a free edge immediately in front of the hind-limbs; thus the tail-fold of the amnion destroyed by the operation has not regenerated, nor is the allantois visible externally, as it always is in normal embryos of this age. The hind-limbs appear normal; part of the tail at least is present.

The enumeration of the postbrachial nerves in the sections gives the following results : 1 to 6 on each side are in front of the leg-rudiments ; the following seven nerves on each side enter the leg-rudiments. On the right side there is only one incomplete ganglion back of the last leg-nerve, *i. e.*, the fourteenth postbrachial ; the nerve from this cannot be traced. On the left side there are four complete ganglia and myotomes back of the last leg nerve (14 to 17 postbrachial). Thus, as might be expected, the hind limbs are normal, and the cloacal region is strongly affected.

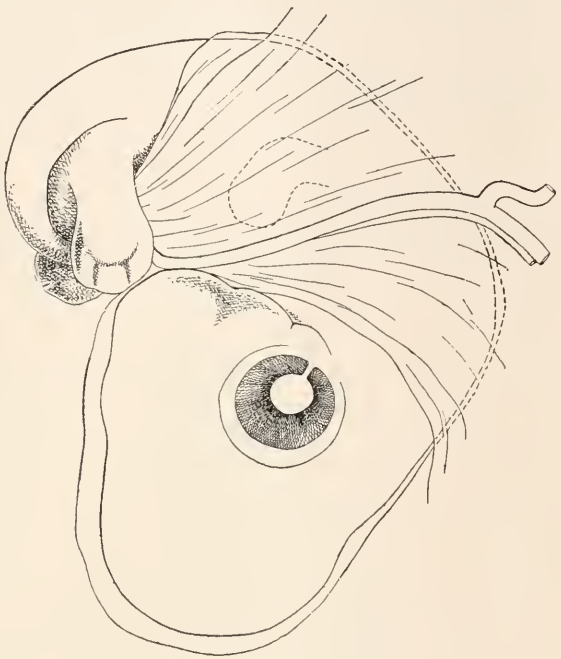


FIG. 7. Defective embryo (Experiment 18).

In the embryo of this experiment the Wolffian ducts have normal relations, but the allantois is thick-walled and undilated ; the entodermal cavity of the latter opens out into a groove in the splanchnopleure, leading back to the hind-gut, which is not closed as it normally is ; in fact in this embryo the cloaca is the only closed region of the hind-gut ; immediately in front of the cloaca it is open ventrally. The stalk of the allantois therefore appears first as a groove on the left side ; followed forwards, this

deepens gradually into a canal that penetrates into a mass of mesoblast connected with the somatopleure, and excavates a large irregular cavity in this. The mass extends forwards to the tip of the ventricle and ends in a bifurcated extremity. This represents the allantois, the main mass of which is composed of loose, very vascular mesenchyme forming an appendage to the somatopleure on the left side. On the right side the mesoblast of the somatopleure in the allantoic region is much hypertrophied and forms a large free lobe without any entodermal contents.

It is remarkable that the allantoic rudiments should show such considerable power of growth in the absence of the usual stimulus of internal pressure, which is precluded by the open groove-like connection with the intestine.

Experiment 24.

The hind end of an embryo was cauterized with the aim of destroying the incipient tail-bud and the region of the hind-

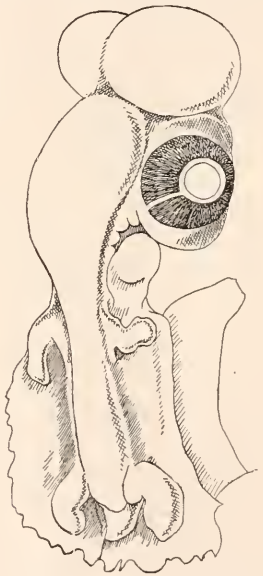


FIG. 8. Defective embryo (Experiment 24).

limbs. The egg was reopened 48 hours after the operation. The vascular area covered about two fifths of the yolk, and appeared to be entirely normal.

Fig. 8 represents the resulting embryo from the dorsal surface; the rudiments of the hind-limbs are apparently as well developed as those of the anterior limbs, and the posterior portion of the trunk is separated from the anterior by a sharp depression. The tail turns to the left, and tapers to an end beneath the left hind-limb; part of its course is hidden by a fold of the somatopleure, as shown in the figure. The depression evidently represents the anterior limit of the injury.

This embryo was sectioned. On examination it was found that, although the region behind the depression has the usual structure of the postanal region of the body, its neural tube has no connection with that of the trunk, and the notochord is absent in the anterior third. Mesoblastic somites occur in this region, but the main part of its substance is made up of loose vascular mesenchyme.

It is plain, then, that this part has not regenerated in the sense that it has grown out from the embryo; and there remains only the assumption that its parts have formed from embryonic tissue remaining in this region after the cauterization. The absence of the notochord throughout almost all of the tail shows that the destruction due to cauterization was very extensive; its absence beneath the neural tube for a considerable distance shows, that the neural tube has formed secondarily in this region; because any operation, that would destroy the notochord, would necessarily destroy the neural tube. Farther, the relation of the Wolffian ducts and allantois to the cloaca are abnormal, and the hinder portion of the intestine has no connection with the anterior portion; all of which shows that there was originally complete destruction of tissues through to the entoderm in the anterior part of the cauterized area. There seems to have been in this case a reorganization of the embryonic tissue of the caudal region.

On the left side I find ten postbrachial ganglia back to the region of the defect. Thus at least four segments of the normal hind limb-region are included, viz., 7, 8, 9, 10. The last is small, representing only a fraction of the normal ganglion. On the right side there are eleven full-sized postbrachial ganglia. Thus there are at least one and one half more metameres on the right than on the left side, and this tallies with larger size of the right hind limb and with the operation diagram.

The only interpretation of this result is that the needle destroyed a transverse section of tissue and separated the tail-rudiment from the rest of the embryo, at the same time causing some injury to the tissue of the tail; secondary union then took place. The tissue along the line of junction is peculiar in many respects, including much material, probably remnants of cauterized parts, that takes the plasma stain.

Experiment 187.

In this experiment the operation was made at a stage of about 24 hours; the medullary plate was just formed and the primitive streak was yet long; probably 3 to 5 somites were present (Fig. 9). Three spots were marked with an electrode, one behind the other in the middle line towards the posterior end of the primitive streak. The three spots were at first separate, but later examination showed them running together; so that all of the

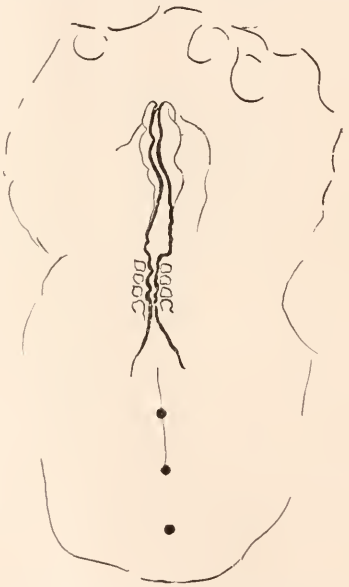


FIG. 9. Operation diagram for experiment 187; drawn from a blastoderm of the same lot of eggs used in this experiment; preserved at the time of the operation.

axial structures were destroyed in this region. The embryo was allowed to develop two days longer and was then preserved.

It will be seen (Fig. 10) that this operation has taken us in front of the vitelline arteries; having been performed before the development of the arteries, it was possible for the rudiment of the vascular system to readjust itself to the new conditions, and establish a vitelline circulation. The vitelline arteries are, however, in about their normal position, *i. e.*, about opposite the 20–22 somites, hence behind the embryo in this case. It is difficult to see why they should have this position and not be shifted farther forward, if the view of His, that the main blood vessels develop from the paths of least resistance in the primitive vascular network, be true.

On the right side of this embryo there are 14 spinal ganglia, and on the left 14 complete and part of the fifteenth; but as, in the fowl, the first two cervical nerves lack ganglia, there are present 16 spinal nerves on the right side, and 17 on the left.

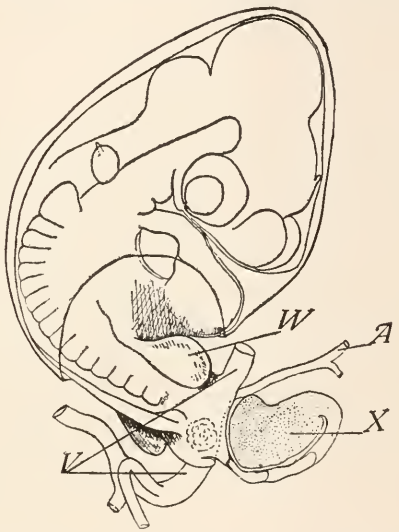


FIG. 10. Defective embryo (experiment 187). The drawing was made from the stained and cleared specimen. *A*, vitelline artery; *V*, vitelline veins; *W*, wing bud; *X*, sac filled with blood, behind the embryo in the prolongation of its axis; this has no connection with the vessels of the embryo.

The normal innervation of the wing is from the fourteenth to the sixteenth spinal nerves. It would thus appear, on the evidence of the nerves, that the operation has destroyed everything back of the wing somites. The wing-buds have a development that appears perfectly normal for this stage.

On the right side there are nineteen myotomes, and on the left twenty and a half. Fig. 10 shows the myotomes of the right side as they appeared in an entire mount of the stained embryo in oil; only 16 can be counted. But sections show, that the large anterior one is really three, and the nineteenth is represented by the mesoblast behind the last complete somite. If we reckon the three anterior somites as cephalic, this leaves sixteen trunk myotomes, and the enumeration agrees with that of the nerves. On the other side of the embryo there is distinctly one more cephalic myotome than on the right side; if, then, we reckon four as the number of cephalic myotomes on this side,[1] there are sixteen and a half trunk myotomes, a result that agrees with the enumeration of the nerves.

The organs in this embryo, as in the others described, have developed normally as far as the cut surface. The Wolffian ducts are enlarged by internal pressure. There has been no compensatory growth and no regeneration, if we except the notochord.

General Discussion.

The chief value of these experiments consists in the fact that they clear the ground for farther researches of a more definite character. It is probable that the results obtained will hold in general for the Amniota, and they may, therefore, be of value in the interpretation of teratological phenomena. Results that I have obtained from destruction of large portions of the brain in young embryos have convinced me that great caution must be exercised in interpreting conditions associated with anencephaly. It should be possible, and I am convinced that it is possible, to produce these various conditions experimentally. Until this is done it seems to me that conclusions, based on teratological material, as to the trophic value of the embryonic nervous system are debatable.

The following questions definitely raised by the experiments just described may be answered here in part:

1. *As to Regeneration.* — The only organ showing evidence of regeneration is the notochord. In all of the embryos described,

[1] Froriep recognizes four cephalic myotomes in the chick; the most anterior, however, becomes rudimentary at a very early stage, and soon disappears; this condition seems to have been attained on one side in this embryo, but not on the other.

the notochord extends a short distance back of the neural tube and its end is very protoplasmic; its further extension in each case is definitely limited by the surface of operation, against which it is pressed (Fig. 5, *A*). One receives the impression that its growth has been stopped by the mechanical hindrance.

One does not expect a vertebrate to show axial regeneration of the trunk, but in certain vertebrates the tail may regenerate. What is the condition in the chick embryo? No. 18 is the only case cited in which any of the caudal somites were left uninjured. In this case there was no regeneration, so far as could be judged.

The very first experiments that I performed on chick embryos were made to determine whether or not the limb-buds might regenerate. The operations were limited to the wing-buds. I found that it was possible to amputate the right one close to the body at a time when its width was equal to or greater than its length, and even later (four or five days); the wound in the amnion might close, and the amputated part remained in the amniotic cavity as evidence of the operation. In the only cases in which the embryo lived for any considerable period after the operation, there was absolutely no sign of regeneration, though the wing-bud of the opposite side increased in bulk several times.

So far as I know, the only evidence, that the organs of the chick embryo possess any different power of regeneration from those of the adult, consists of the above observations on the notochord (tissue regeneration) and of Barfurth and Dragendorff's[1] observation on the regeneration of the lens of the eye from the edge of the optic cup. The last depends on observations on a single case, and in this case the extent of injury to the eye was doubtful. Until the result is confirmed, I believe we are justified in passing it over.

There would remain, then, the general conclusion, subject only to the qualifications already noted, that the embryo of the chick possesses no greater power of regeneration than the adult.

2. *As to the Somites.* — Somites always retain their normal numerical value. But the experiments were not adapted to analyze very accurately the numerous problems presented.

[1] Dietrich Barfurth and O. Dragendorff, "Versuche über Regeneration des Auges und der Linse beim Hühnerembryo," Anat. Anz., Ergänzungsheft zum XXI. Bd., 1902.

Thus the operation destroyed in each case not only definite somites, but also all posterior to the first one injured, and the body wall lateral to the somites. With more refined methods, it may be possible to eliminate single somites from within the series, and to avoid injury to adjacent tissues. Such a technique would enable one to analyze many of the problems offered by the somites: to determine, for instance, the exact part played by them in development of the limbs, the order of origin of the most anterior somites, etc. Such problems are now being studied with partial success, and the results will be published later.

UNIVERSITY OF CHICAGO,
March, 1904.

THE RELATIONS OF THE ANTERIOR VISCERAL ARCHES TO THE CHONDROCRANIUM.

W. K. GREGORY.

The articular relations with the chondrocranium of the upper and lower jaw-cartilages and the hyomandibular, as typified in *Ceratodus*, in *Squalus*, and in *Notidanus*, are in themselves of course generally understood, but comparison of the current definitions and usages of the corresponding terms "autostylic," "hyostylic," "amphistylic" reveals considerable discrepancy, which is highly confusing to the general student; hence the present endeavor to standardize these terms and to give, as far as needed, their synonymy.

From the analysis necessary for the accomplishment of this purpose it has become evident that all the current definitions of "hyostylic," "autostylic" and "amphistylic" are in one way or other unsatisfactory, and that if these conceptions are to retain anything more than historic interest they will have to be extended to include the relations to the chrondrocranium not only of the hyomandibular but also of the hyoidean arch as a whole and of its distal or ventral half, the "hyoid" or ceratohyal. By this extension we are enabled: first, to apply separate and clearly diagnostic terms to the suspensorial conditions of the very phylogenetically separated groups Dipnoi (autostylic) and Holocephali ("holostylic"), hitherto lumped together under the single term "autostylic"; second, to differentiate under the generic concept "hyostylic" four specifically well-marked modes, (*a*) the "hyostylic proper" of typical sharks, (*b*) the "amphyostylic" of *Notidanus*, (*c*) the "euhyostylic" of most rays, (*d*) the "methyostylic" of the teleostomes; third to group all the modern types of suspensorial conditions under the term "cænostylic" in contrast with the ancestral type here called "palæostylic," which is only a few steps below Gadow's[1] suggested form, the "simple autostylic."

[1] "On the Modifications of the First and Second Visceral Arches, etc.," *Philos. Trans.*, Vol. 179 (1888) B, p. 459.

TABLE A.

	PALATOQUADRATE. Proximal or dorsal moiety of "first" visceral or oral arch.	SECOND VISCERAL ARCH.	HYOMANDIBULAR. Proximal or dorsal moiety of "second" visceral or hyoidean arch	HYOID OR CERATOHYAL. Distal or ventral moiety of "second" visceral arch.	DESCRIPTIVE TERMS. Huxley, '76.	Weidersheim, '88. (Parker's.)	Zittel, 1902. (Eastman's.)	Gadow, '88.	A. S. Woodward, '98.	Preferable Term.
Hypothetical Ancestor.	Preceded by "preoral" arches, gill bearing, not separated by distinct joint from distal (ventral) or mandibular portion. Slight or no cranial attachments.	Intact, and without midjoint.	Not suspensorial	Not suspensorial.						**Palæostylic.**
Chlamydoselachus. (*Fide* Garman '85.) (Fig. 2.)	Loosely articulating with cranium. With postorbital process.	Intact.	Very large; suspensorial; attached distally to jaws, pointing backward, movably articulating with cranium.	Retaining primitive connection with distal end of hyomandibular, closely applied to and supporting mandible.		Hyostylic.	Hyostylic	Amphistylic.	Hyostylic	**Hyostylic.**
Cestracion.	Closely articulating far forwards with cranium. Deep posteriorly.	Intact.	Of medium size; attached to cranium proximally by ligaments; of considerable suspensorial value; attached to junction of quadrate and mandible.	Articulating with distal end of hyomandibular, attached anteriorly to and supporting mandible.	Amphistylic. Low form of hyostylic.			{Autostylic Amphistylic.}	Hyostylic.	**Hyostylic.**
Notidanus. (*Fide* Gadow '88.) (Fig. 4.)	Closely articulating with cranium, especially by "otic" process, much deepened posteriorly; inner joint with mandible incomplete. (Gadow.)	Intact.	Slight; proximally attached to cranium by ligaments, of indifferent suspensorial value.	Retaining primitive connection with the distal end of the hyomandibular, attached by ligament to inner superior border of mandible; of slight or no suspensorial value.	Amphistylic.			Autostylic.	Hyostylic.	**Amphyostylic.**
Typica Sharks and *Squatina.* (Fig. 1.)	Loosely attached to cranium.	Intact.	Movably attached to cranium; distally supporting quadrate region, mandible and hyoid.	Retaining primitive connection with distal end of hyomandibular, attached anteriorly to and supporting mandible.	Hyostylic	Hyostylic.	Hyostylic.	{Amphistylic. Hyostylic}	Hyostylic.	**Hyostylic.** *Sensu strictu.*

Typical Rays. (Fig. 3.)	Free from cranium.	Broken up.	Enlarged, the sole suspensorium	Proximal end, partly by metatrophy of hyomandibular, articulating in different forms at different points along the hyomandibular, finally reaching the cranium, even gaining a distinct articulation with it; widely separated from the jaws; gill bearing. (Wiedersheim, Gadow.)	Hyostylic. Extreme form of hyostylic.	Hyostylic	Hyostylic.	Hyostylic.	Hyostylic.	**Euhyostylic.**
Polypterus. (Fig 6.)	Forming cartilaginous basis of palato-pterygoquadrate series as in teleosts.	Broken up.	Large, with broad articulation with cranium in sphenotic region, supporting operculum; buttressing metapterygoid and thus, indirectly, the quadrate. Capped by a pharyngohyal (?).	Modifying primitive connection with hyomandibular through intervention of the interor stylo-hyal; aiding in support of mandible.	Hyostylic.				Hyostylic	**Methyostylic.**
Acipenser.	Free from cranium.	Broken up.	Enlarged, the sole suspensorium through its separate symplectic segment.	Reduced, beginning the dorsad migration along the hyomandibuliar towards the cranium	Hyostylic.	Hyostylic.	Hyostylic		Hyostylic.	**Methyostylic.**
Typical teleosts. (Figs. 5, 7, 8.)	Forming cartilaginous basis of palato-pte ygoquadrate series.	Broken up	Articulating with cranium in sphenotic region, distally supporting quadrate through the metapterygoid, symplectic and preoperculum; carrying the whole opercular apparatus, which in turn braces the quadrate.	Modifying its hyomandibular connection by intervention of interhyal. Functioning chiefly in support of tongue and branchiostegal bars.	Hyostylic.	Hyostylic.	Hyostylic.		Hyostylic.	**Methyostylic.**
Amphibia. (*Fide* Gadow.) (Fig. 10.)	Partially fused with cranium, forming cartilaginous basis of dermal bones.	Broken up.	Proximal part functioning as stapes (Gadow).	The proximal end having lost its connection with the hyomandibular generally becomes attached to the cranium as the stylohyal (Gadow).	Autostylic, Amphistylic (of larva).			Autostylic.		**Autostylic.**
Ceratodus. (*Fide* Huxley, '76, Gadow, '88.) (Fig. 9.)	Entirely fused with cranium, forming "suspensorial cartilage" posteriorly.	Broken up.	Reduced, united with cranium anteriorly, bearing the operculum, conical distal portion homologous with the symplectic (Huxley).	Connected by ligament with reduced hyomandibular.	Autostylic.	Autostylic.	Autostylic.	Autostylic Holostylic.	Autostylic.	**Autostylic.**
Chimæra. (See p. 61.) (Fig. 11.)	Entirely fused with cranium. Very short antero-posteriorly.	Intact.	Entirely separated from palato-quadrate and mandible, capped by small pharyngohyal.	Retaining primitive connection with hyomandibular.	Autostylic	Autostylic.	Autostylic	Autostylic Holostylic.	Autostylic.	**Holostylic.**

The foregoing table summarizes the relations in various forms of the first and second visceral arches to the chondrocranium, and gives the descriptive terms used by authors and the terms here adopted.

Upon this analysis the following definitions may be based.

PALÆOSTYLY: "First" visceral or mandibular and "second" visceral or hyoidean arches retaining in large part their primitive function as gill bearers, subequal in size, with slight or no connections with the chondrocranium; preoral gill arches. Hypothetical.

CÆNOSTYLY: "First" and "second" visceral arches modified in correlation with feeding, unequal in size, one or more of the elements attached to the cranium. Preoral arches changed in function (=labial cartilages and trabeculæ cranii.)

A. HOLOSTYLY[1]: Platoquadrate fused with chondrocranium; second visceral or hyoidean arch intact, non-suspensorial and free from the cranium. Holocephali.

B. AUTOSTYLY[2]: Palatoquadrate fused with chondrocranium; second visceral arch broken up and non-suspensorial, the hyomandibular reduced and united with the cranium. Dipnoi, Amphibia.

C. HYOSTYLY: Palatoquadrate articulating with chondrocranium, hyomandibular more or less suspensorial.

a. Hyostyly proper.[3] Second visceral arch intact, the hyomandibular and hyoid segments forming a movable suspensorium for the upper and lower jaws. Most sharks, *Squatina*.

b. Euhyostyly.[4] Second visceral arch broken up, the dorsal segment (hyomandibular) forming the sole suspensorium, the distal regment (ceratohyal) secondarily, free from all connection with the jaws, functioning solely as a gill bearer. Most rays.

[1] "Holo," in allusion, either to "Holocephali," or to the fact that probably since early Palæozoic times the palatoquadrate and the cranium have formed a continuous whole.

[2] The term "autostylic," hitherto applied to both Dipnoi and Holocephali is here restricted to apply only to the former.

[3] "Hyostyly proper" because the ordinary sharks furnish the traditional type of this condition.

[4] "Euhyostyly," as a progression upon or development of the "hyostyly proper."

c. Amphyostyly.[1] Second visceral arch intact, of slight suspensorial value, much smaller than first arch; palato-quadrate deep posteriorly, articulating by its "otic process" with the chondrocranium. *Notidanus*, *Pleuracanthus*.

d. Methyostyly.[2] Second visceral arch broken up (*i. e.*, with the component segments more or less shifted out of their primitive sequence or relations); symplectic, metapterygoid, pre- and interopercular when present assisting the hyomandibular in the support or bracing of the quadrate and mandible.

The value and permanence of this classification will depend on whether the hyomandibular of teleostomes is homologous, as generally supposed, with that of elasmobranchs. This has been called into question by Pollard ('94) but as his conclusions have not been adopted by subsequent authorities it seems best to accept the traditional view that these elements are truly homologous in the two groups.

The application of these terms in classification and phylogeny is illustrated in the diagram on page 60.

Previous Definitions.

Huxley. — The terms "autostylic," "amphistylic," "hyostylic" were first used by Huxley in his paper of 1876 on *Ceratodus*.[3] The passage in which "autostylic" is defined is as follows (*op. cit.*, p. 40):

"The part of the palato-quadrate cartilage [of *Ceratodus*] which is united with the skull, between the exits of the fifth and second nerves, answers to the "pedicle of the suspensorium" of the amphibian, while its backward and upward continuation onto the periotic cartilage corresponds with the otic process. As in the Amphibia and in the higher Vertebrata, the mandibular arch is thus attached directly to the skull by that part of

[1] "Amphyostyly"; this term retains the element "amphi," so long used (in *amphistylic*) of these forms, while clearly indicating its subordination under hyostyly.

[2] "Methyostyly," in allusion either to the prominence of the *meta*pterygoid in the suspensorium, or to the fact that methyostyly represents a morphological advance upon earlier modes.

[3] *Proc. Zool. Soc. Lond.*, 1876, pp. 24–59. "The Scientific Memoirs of Thomas Henry Huxley," Vol. IV., 1902, pp. 84–127.

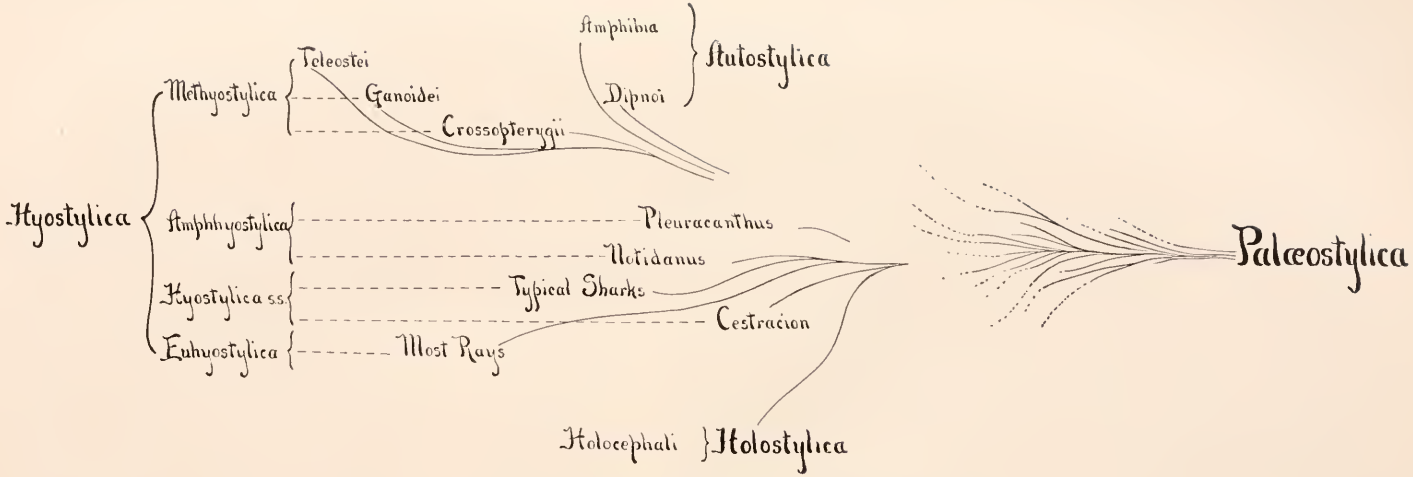


FIG. 1. Application of the newly defined terms to the phylogeny of the fishes.

its own substance which constitutes the suspensorium. It may thus be said to be *autostylic*.

"Among fishes the only [other?] groups which possess an autostylic skull or in which the dorsal end of the mandibular arch is continuous with the cartilage of the brain case are the Chimæroids and the Marsipobranchii."

In this definition of autostyly attention is centered solely upon the relations of the *first* arch with the skull. Huxley notes that in the autostylic *Ceratodus* the hyomandibular is reduced and fused with the skull, but he also uses "autostylic" for *Chimæra* in which the hyomandibular is separate (see above, Table A). For comparison with "hyostylic" and "amphistylic" we may summarize Huxley's description as follows:

AUTOSTYLIC SKULL: *Mandibular or first visceral arch attached to the skull solely by its own dorsal moiety, the palatoquadrate, which is continuous with the skull.*

Huxley describes the HYOSTYLIC and AMPHISTYLIC conditions as follows (p. 41):

"In all other Fishes, except the Marsipobranchii, the mode of connection of the mandibular arch with the skull is different from that which obtains in the Chimæroids and the Dipnoi. The palatoquadrate cartilage is no longer continuous with the chondrocranium . . . but is, at most, united with it by ligament. Moreover the dorsal element of the hyoidean arch, or the hyomandibular, usually attains a large size and becomes the chief apparatus of suspension of the hinder end of the palatoquadrate cartilage with the skull. Skulls formed upon this type, which is exemplified in perfection in Ganoidei, Teleostei, and ordinary Plagiostomes, may therefore be termed *hyostylic*.

"But though the typical forms of autostylic and hyostylic skulls, as exemplified, *e. g.*, by a Sturgeon, a Pike, and a Dogfish or Ray, on the one hand, and *Chimæra*, *Ceratodus*, and *Menobranchus* on the other, are thus widely different, certain Plagiostomes present a condition of the cranium which tends to connect the two by a middle form, which may be termed *amphistylic*."

"In the amphistylic skull the palato-quadrate cartilage is quite distinct from the rest of the skull; but it is wholly, or almost wholly, suspended by its own ligaments, the hyomandibular being small and contributing but little to its support. The embryo amphibian is amphistylic before it becomes autostylic; and, in view of certain palæontological facts, it is very interesting that the link which connects the amphistylic with the ordinary Selachian skull is that of *Cestracion*."

Huxley's conception of hyostyly and amphistyly may be restated as follows:

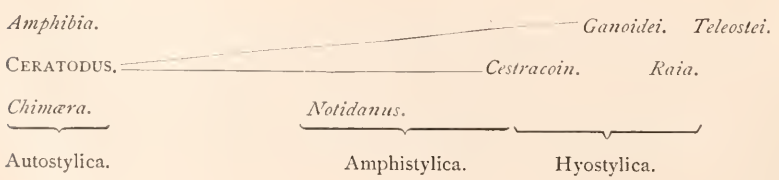
Hyostylic Skull: *Mandibular or first visceral arch not continuous with the skull in its dorsal portion (the palatoquadrate); the hinder end of the palatoquadrate is chiefly connected with the chondrocranium by means of the hyomandibular or dorsal portion of the hyoidean arch.*

Amphistylic Skull: *Mandibular, or first visceral arch attached to the skull wholly or almost wholly by means of its dorsal portion the palatoquadrate, not however by fusion or continuity, but by ligaments; the hyoidean arch contributes but little to its support.*

The Huxleyan conception of amphistyly is that it is in some respects a "middle form," resembling autostyly in the suspensorial self-sufficiency of the first visceral arch, but resembling hyostyly in the lack of confluence of the palatoquadrate with the skull. A similar use of the prefix "amphi" occurs in "*Amphitherium*," which genus was at first supposed to combine mammalian and reptilian characters.

The element "stylic" from *στῦλος*, a pillar, evidently refers to the quadrate region in its architectural relation to the skull. The skull of *Chimæra* seems to have been regarded by Huxley as primitive (so far as I can determine from a careful study of the entire article) and hence in an autostylic skull the palatoquadrate must have been conceived as belonging to the skull: therefore the forces acting upon the jaws in eating would be transmitted to the skull chiefly through the quadrate, *its own pillar* (hence auto, stylic), whereas in the typical hyostylic skull these forces would be transmitted chiefly through the pillar of the hyoidean arch (the hyomandibular) — hence "hyostylic."

Huxley's conception of the phylogenetic relations of the three types of cranial structure are expressed in the following diagram (*op. cit.*, p. 45):



The left-hand column (autostylica) includes the (to Huxley) more generalized types, the most primitive, *Chimæra*, standing at

the bottom of the column; progressive stages of skull structure are represented in the middle and right-hand columns. The cranial plan of *Ceratodus* is regarded as representing the ancestral condition of both *Cestracion* and the Ganoids. *Cestracion*, classed with *Notidanus* as an amphistylic type, is regarded also as transitional, a low form of the hyostylic type.[1] The diagram confirms the inference that Huxley conceived the palatoquadrate as originally a part of the skull, which gradually became constricted off as in *Ceratodus* and finally freed from it entirely as in *Raia* and the Teleosts.

Wiedersheim (*Parker's translation*, '97). —

"The palatoquadrate is usually only united to the basis cranii by ligaments, but in the Chimæroids. . . it becomes immovably fused with it, whence their name of Holocephali. In the Sharks and Rays the palatoquadrate is not directly united to the skull, but is suspended from it by the *hyomandibular*. . . . In this case the skull may be described as *hyostylic*, to distinguish it from *autostylic* skulls, in which the hyoid takes no part in the suspensorium" (*op. cit.*, p. 75).

Comments: (1) "Hyoid" here refers to the hyoidean arch, not to the hyoid or ceratohyal. (2) In general it would be less confusing to say that it is the *mandible* rather than the palatoquadrate which is suspended from the hyomandibular, and that the palatoquadrate rests chiefly upon and is fastened to the mandible.

v. Zittel, 1903 (*Eastman's edition*). —

"In the Holocephali the palatoquadrate and hyomandibular fuse together and with the cranial capsule. The mandible thus becomes autostylic, *i. e.*, articulates directly with the cranium" (*op. cit.*, p. 11).

Comments: (1) The statement in regard to the hyomandibular seems incorrect. In a specimen of *Chimæra collei* kindly loaned to me by Professor Bashford Dean the hyomandibular is seen to be a large independent element (see Fig. 11) serially homologous with the epibranchials as in Selachii, and tipped with a reduced pharyngohyal, both being free from the chondrocranium. (2) To apply the term autostylic to the *mandible* is to introduce a new element of confusion in a matter already sufficiently complicated.

[1] On page 43 of Huxley's memoir the *Cestracion* skull is referred to as a low form of "autostylic type," but examination of the context and of the diagram cited show that this is probably a misprint for "hyostylic."

Parker and Haswell (1897). —

"In some fishes the hyomandibular articulates above with the auditory region of the cranium while the jaws are connected with its ventral end. We may thus distinguish two kinds of *suspensorium* or jaw-suspending apparatus, a *mandibular suspensorium*, furnished by the quadrate, and a *hyoidean suspensorium* by the hyomandibular; in the former case the skull is said to be *autostylic, i. e.*, having the jaw connected by means of its own arch, in the latter it is called *hyostylic*; in a few cases an *amphistylic* arrangement is produced by the articulation of both mandibular and hyoid arches with the skull" (*op. cit.*, p. 71).

On page 161 we read that in *Hexanchus* and *Heptanchus*: ". . . there is a prominent post-orbital process of the palatoquadrate for articulation with the post-orbital region of the skull (amphistylic arrangement)."

The definitions cited may be criticised on several grounds:

1. There are several ambiguities latent in these sentences which experience proves to be exceedingly puzzling to the student: (*a*) "jaw" and "jaws" thus used require the most careful analysis to determine which jaws, upper or lower, are intended; (*b*) a *mandibular suspensorium* is no doubt equivalent to "a suspensorium furnished by the mandibular or first visceral arch"[1] but if "mandibular" be mistakenly interpreted as referring to the mandible the very pith of the definition is lost; (*c*) *suspensorium* or jaw-suspending apparatus is understood first with reference to the lower jaw, then to the upper; (*d*) "hyoid" arch is used on this same page (71) as referring to the whole second visceral arch, but on page 161 "hyoid arch" is used of the ceratohyal or hyoid only.

2. The definition of autostylic does not exclude *Notidanus* (which Parker and Haswell themselves call "amphistylic") because in it the hyomandibular takes so small a share in suspension that the suspensorium may be said to be "furnished by the quadrate."

3. The definition of amphistylic as implying the articulation of "both mandibular and hyoid arches with the skull" is exceedingly imperfect. As shown above (Table A, pp. 54, 55) the articulation of both mandibular and hyoidean arches with the skull is not diagnostic of amphistyly since on the one hand both arches

[1] Especially since "mandibular" is used in that sense at the top of the same page.

articulate with the skull in many hyostylic types, and on the other hand in the amphistylic *Notidanus* both arches do not articulate with the skull since the hyomandibular is connected with it only through ligaments (Gadow, '88, Pl. 71, Fig. 1, A).

Gadow (1888). —

Gadow apparently uses the terms in their etymological significance, rather than with the limitations imposed by established usage. Thus he speaks (p. 455) of the "autostylic condition of the *Notidanidæ*" (the "amphistylic" of authors) and applies the same term to the Amphibia (p. 481) and the Dipnoi (p. 459), evidently having in mind the self-sufficiency in all these cases of the first arch as its own suspensorium; so too, "simple autostylic" (p. 459) apparently refers to the ancient and generalized condition from which the modern modes have been derived (cf. "palæostylic," proposed above) and in which the oral arch had not yet begun to borrow support from the hyoidean arch. Again Gadow applies "holostylic" (p. 458) to *Chimæra*, *Ceratodus* and *Cestracion* evidently with reference to the functional ligamentous union of the upper jaw with the cranium in *Cestracion* and to its fusion therewith in *Chimæra* and *Ceratodus*. "Amphistylic" in its etymological sense serves to describe the conditions in "most selachians" (p. 481), in which *both* oral and hyoidean arches are suspensorial, while etymologically the rays are preeminently "hyostylic" (p. 481) since the dorsal element of the *hyoidean* arch, the hyomandibular, is the sole suspensorium (cf. our "euhyostylic," p. 56, *supra*).

But while this bold and highly suggestive use of terms has finally bred in me several clarifying ideas I cannot deny that formerly, wishing to refer to isolated passages of Gadow's work, I experienced a most baffling uncertainty as to his meaning.

Smith Woodward (1897). —

"Among the fishes existing at the present day there may be observed two distinct plans of cranial structure, between which no definitely intermediate conditions can be recognized. In *Chimæra*, *Protopterus*, *Ceratodus* and their allies, the upper segment of the mandibular arch is directly fused with the chondrocranium, while the corresponding segment of the hyoid arch is atrophied or absent; in the Elasmobranchs and the so-called "Ganoidei" and "Teleostei" the same elements are loosely articulated with the chondrocranium, the upper segment of the hyoid arch forming a

movable suspensorium. The first condition is now commonly known as the *autostylic*, and the second as the *hyostylic*."

Comments. — This passage is perfectly clear and illuminating. It adheres closely to the essential features of Huxley's conceptions, and it makes a decided advance beyond them not only in stating clearly that between modern autostyly and hyostyly "no definitely intermediate conditions can be recognized," but also in implicitly reducing the amphistylic mode to its true place as a special phase of the hyostylic — a suggestion which has been followed in the present paper (see above, pp. 57, 59).

But not even these definitions, I think, will survive. They fail to emphasize the differences between the autostyly of Dipnoi and the autostyly (here termed "holostyly") of Chimæroids; nor do they indicate that by taking also the distal segment of the hyoidean arch into account we may place the whole subject upon a new, and apparently phylogenetic basis.

In conclusion I desire to thank Professor Henry Fairfield Osborn and especially Professor Bashford Dean for various courtesies and suggestions.

REFERENCES.

Dean, B.
'95 Fishes, Living and Fossil, 8vo, 1895.

Gadow, H.
'88 On the Modifications of the First and Second Visceral Arches, etc. Philos. Trans. Lond., Vol. 179 (1888) B, pp. 451–485, pl. 71–74.

Garman, S.
'85 Chlamydoselachus anguineus Garm. — A living species of Cladodont Shark. Bull. Mus. Comp. Zool. Cambridge, Mass., 1885, pp. 1–35, Pl. I.–XX.

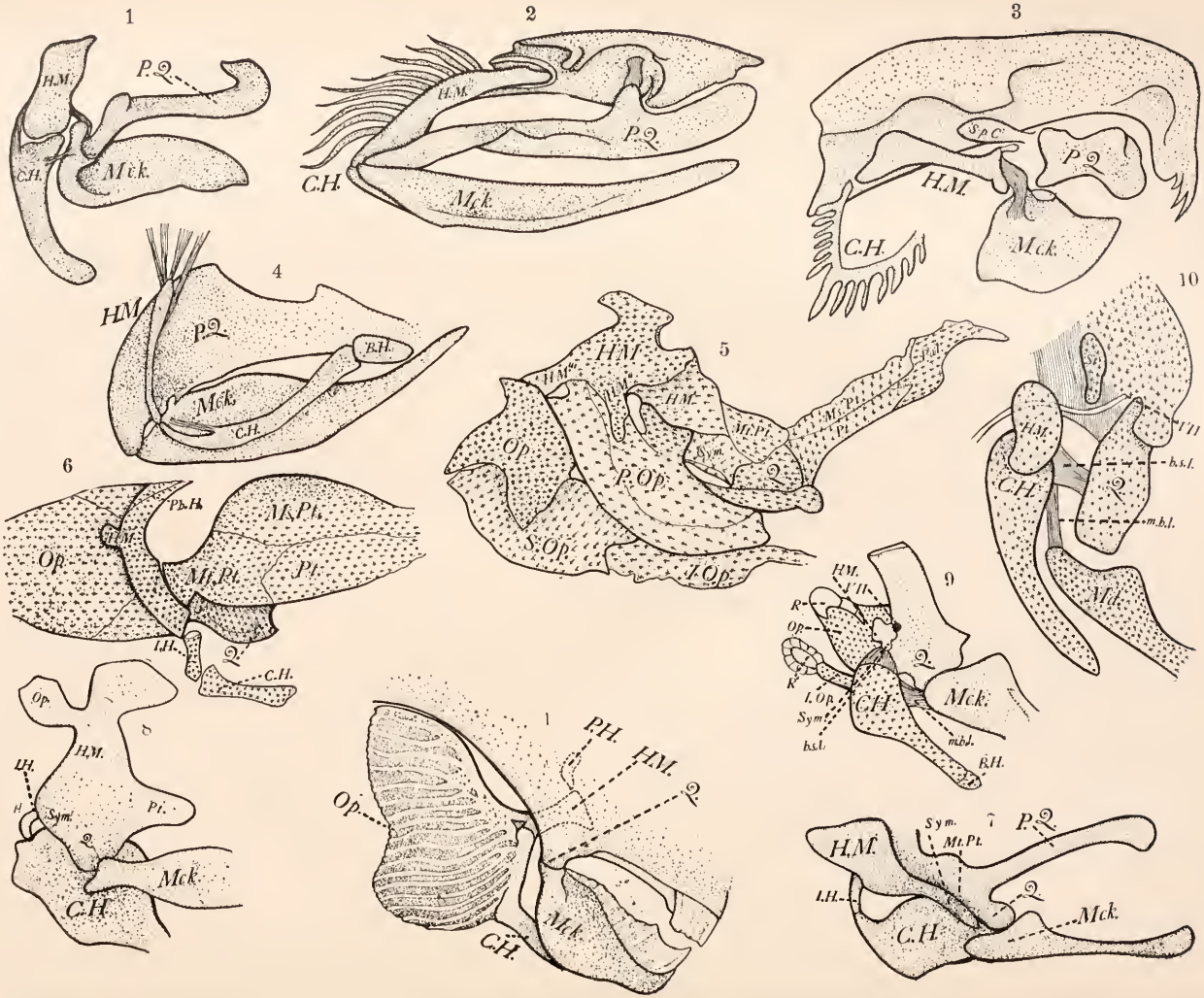
Huxley, T. H.
'76 On Ceratodus fosteri, with Observations on the Classification of Fishes. Proc. Zool. Soc. Lond., 1876, pp. 24–59.

Parker and Haswell.
'97 A Text Book of Zoology, Vol. II., 8vo, 1897.

Pollard, H. B.
'94 On the Suspension of the Jaws in Fish. Anat. Anz., Bd. X., 1894, pp. 17–25.

Smith Woodward, A.
'98 Outlines of Vertebrate Palæontology, etc., 8vo, 1898.

Wiedersheim, R. (W. N. Parker, Tr.).
'97 Elements of the Comparative Anatomy of Vertebrates, second edition, 8vo, 1897.


1
H.M.
P.Q.
C.H.
M.c.k.
2
H.M.
P.Q.
C.H.
M.c.k.
3
Sp.C.
P.Q.
H.M.
C.H.
M.c.k.
4
H.M.
P.Q.
B.H.
M.c.k.
C.H.
5
H.M.
H.M.
H.M.
M.Pt.
Ms.Pt.
Pt.
Sym.
Op.
P.Op.
S.Op.
I.Op.
6
Pt.H.
H.M.
M.Pt.
Op.
M.Pt.
Pt.
I.H.
Q.
C.H.
7
Op.
H.M.
I.H.
H
Sym.
Pt.
Q.
Mck.
C.H.
8
P.H.
H.M.
Op.
Q.
Mck.
C.H.
9
H.M.
I.H.
R
Op.
Q.
K
I.Op.
Sym.
h.s.l.
C.H.
Mck.
m.b.l.
B.H.
10
H.M.
I.H.
h.s.l.
C.H.
Q.
m.b.l.
Md.
7
Sym.
Mt.Pt.
P.Q.
H.M.
I.H.
Q.
C.H.
Mck.

v. Zittel, K. A. (C. R. Easman, Ed.).
'02 Text Book of Palæontology, Vol. II., 8vo, 1902.

COLUMBIA UNIVERSITY,
DEPARTMENT OF ZOÖLOGY,
February 10, 1904.

PLATE I.

EXPLANATION OF ABBREVIATIONS.

P.Q., Palatoquadrate.
Mck., Mandible (Meckelian cartilage).
H.M., Hyomandibular (epihyal).
B.H., Basihyal.
C.H., Ceratohyal.
P.H., Pharyngohyal.
Sp.C., Spiracular cartilage.
I.H., Inter- or stylohyal.
Pal., Palatine.
Ms.Pt., Mesopterygoid (entopterygoid).
Pt., Pterygoid.
Mt.Pt., Metapterygoid.
H.M'., Anterior limb of hyomandibular.
H.M''., Median limb of ditto.
H.M'''., Posterior limb of ditto.
Sym., Symplectic.
Q., Quadrate.
P.Op., Preoperculum.
I.Op., Interoperculum.
Op., Operculum.
S.Op., Suboperculum.
VII., Aperture for seventh nerve.
h.s.l., Hyosuspensorial ligament.
m.h.l., Mandibulohyoid ligament.
St., Stapes.

Cartilaginous elements stippled, osseous elements with small crosses.

FIG. 1. "Hyostyly proper." *Centrophorus granulosus.* Embryo. After Gadow.

FIG. 2. "Hyostyly proper." *Chlamydoselachus anguineus.* After Garman.

FIG. 3. "Euhyostyly." *Trygon* sp. After Gadow.

FIG. 4. "Amphyostyly." *Heptanchus cinereus.* Internal view. After Gadow.

FIG. 5. "Methyostyly." Diagram of the relations of the suspensorial and opercular regions in the cod.

FIG. 6. Methyostyly. *Polypterus.* View from within and below. The metapterygoid is supported by the hyomandibular.

FIG. 7. Cartilaginous basis of methyostylic arrangement in the young salmon. After Parker and Bettany.

FIG. 8. Cartilaginous basis of methyostylic arrangement in a larval siluroid. After Pollard. All the cartilages of the suspensorial region seem to have secondarily coalesced.

FIG. 9. "Autostyly." *Ceratodus.* After Huxley. *R*, *R'*, vestigial hyoid rays. The operculum is borne by the reduced hyomandibular.

FIG. 10. "Autostyly." *Proteus.* After Gadow. The stapes represents the upper portion of the hyomandibular (Gadow).

FIG. 11. "Holostyly" *Chimæra collei.*

VARIATION IN BEES — A REPLY TO MR. LUTZ.

EVERETT F. PHILLIPS.

In the December (1903) number of this journal there appeared an article entitled "Comparative Variability of Drones and Workers of the Honey Bee" by Dr. D. B. Casteel and myself in which we concluded that the drones show the more variability, which result we attributed to the effect of the size of the cells on the developing larvæ and pupæ. Mr. Frank E. Lutz in the April (1904) number of this BULLETIN thinks it "well worth while to consider a few points about the paper" and concludes that we have not proven our point. I desire here to point out the errors in his manner of dealing with our measurements and his methods of drawing conclusions and to defend our position.

The fact that in the consideration of abnormal veins we found that "there are 206 irregular drone wings and 30 irregular worker wings or almost seven times as many for drones as for workers" points rather conclusively to our position in regard to comparative variability. Our statements in regard to coloration are also disregarded, evidently because there were no figures given and therefore they were not considered worthy of mention. In another paper[1] I have dealt with the comparative constancy of the color areas of the two sexes and there offer evidence which confirms the position maintained in our paper, although as there used it was intended to prove an entirely different point. In quoting from a letter from Mr. E. R. Root, a high authority in apiculture, it was there stated: "The drones from these queens (imported Italians of pure stock) varied greatly in their markings. Some of their sons would have a great deal of yellow on them, while others would be quite dark. . . . *Bees* (workers) from these queens were all uniformly marked." In variation work but few investigators take the trouble to study more than one character yet here are two which are rather conclusive and on these we would be willing to rest the case.

[1] "A Review of Parthenogenesis," *Proc. Am. Philos. Soc.*, XLII., No. 174; *vide* pp. 277–8.

I will now take up the more definite criticisms of Mr. Lutz. In our paper we gave reasons which to us seemed good why we did not employ the standard deviation method in our work, stating distinctly that "that would be undesirable except with far more measurements"; this could be interpreted only as a desire on our part to avoid a high probable error and no one need "wonder greatly" why we did not employ this simple test since we distinctly stated that we did not think it desirable, giving full reasons for such a decision. It was not our intention to take exception to the methods of the workers in variation for I believe that the results of variation work should be stated in mathematical formulæ where such a thing is possible without too great a "probable error." If our critic is trying to defend such methods under the impression that we combat them, he is laboring under a misapprehension. However Mr. Lutz has seen fit to figure out the standard deviation and probable error for two of our tables and concludes "that the differences between the two sexes, as shown by these data are of no significance." I am still of the opinion that it is unwise to use these methods for so few individuals on account of the large probable error but since this is the way in which our results are questioned let us examine the figures and see if there is not "great danger that, having collected a set of measurements" our critic makes "a show of accuracy that will lead" him "and others astray by reason of careless and insufficient analysis." For ease of reference I give the results of Mr. Lutz.

The argument of Mr. Lutz is that since there is as much difference in standard deviation between various lots of drones as there is between the averages of the standard deviations of drones and workers, our conclusion that drones vary the more is false. But is it not evident that in every case of the drones (except lot II.) the standard deviation or index of variability is greater than that of the workers? I cannot see what bearing the differences in the standard deviation of the various lots of drones has on the question since in every case except one, the drones do vary more than the workers; and this it was that we attempted to prove. In regard to this lot II. we said: "The drones in lot II. were taken from a hive in which there were no drone cells except

HOOKS ON HIND WING.

Lot.	Number of Specimens.	Standard Deviation.	Probable Error [1]
		Drones.	
I.	50	2.1548	0.1453
II.	100	1.5435	0.0736
III.	100	1.7716	0.0845
IV.	100	1.6486	0.0786
V.	50	2.0988	0.1416
VI.	98	1.9377	0.0934
		Workers.	
I.	50	1.5223	0.1027
II.	350	1.5564	0.0397
III.	100	1.5523	0.0740

VEIN *R*.

Lot.	Number of Specimens.	Standard Deviation.	Probable Error.
		Drones.	
I.	50	2.4023	0.1620
III.	100	2.9598	0.1412
V.	50	2.2517	0.1519
		Workers.	
I.	50	1.5637	0.1055

possibly a very few in the corners of the frame or near the top bar of the frame since all the combs were made on what bee-keepers call foundation and the cells were uniformly of worker size. These drones show the least variation since they were all hatched under the same conditions." Even if we add the probable error to the standard deviation of workers and subtract it from that of the drones this result holds except in lots II. and IV. and it would seem that from these figures (made by Mr. Lutz himself) that our results are most strongly confirmed.

The additional criticism is made that we lumped the different lots together because they seemed to be alike " when really their only claim to homogeneity is that they are of the same sex and all bees — Italians, hybrids, 'peculiar strains,' et al., from central Ohio to eastern Pennsylvania being jumbled together." This

[1] In discussing the average standard deviation of this table Mr. Lutz takes the average of the probable error for the lots of 50 and 100 without taking into consideration that the probable error should be smaller for 500 than for these lots. Surely since all the lots show the same greater variability of the drones (except lot II. as explained later) the probable error is considerably smaller than that given by Mr. Lutz.

was done only in the case of our figures concerning the ratios between the veins M_2 and m where a careful examination showed us that these ratios did not vary according to lots but from the criticism it might be inferred that we had committed this "grave error" throughout the work. Just how Mr. Lutz could know, since the ratios for the individual wings were not published, that we had "jumbled together" some measurements in an unjustifiable manner is still a mystery but I am convinced that his criticism is unjust, having carefully examined the ratios with this very criticism in mind at the time of the preparation of our paper.

At the close of his article our critic says: "It is also probably unnecessary to remark that, even if it turns out that the greater variability of the drones can be established, their proof of their theory to account for this difference seem rather unsatisfactory." I think it is shown, by us and by Mr. Lutz, that the drones do vary the more and our theory of the cause, based as it is on careful investigations of the habits of the bee, must be controverted by more observations of an equally careful nature. Too often students of variation work on forms, concerning the habits of which they know nothing, and conclusions are reached which would be modified if causes were looked for in the habits, but I feel free to state that we are not open to that criticism.

By omitting the parts of our paper in which we explain our stand regarding the variability being "due to chance," Mr. Lutz would make out that we do not know that all chance is in accord with some mathematical formula. On this point we stated: "It may be argued that variation according to chance is but a way of stating our ignorance of the true law, but if there is a law for this variation it is certainly very obscure, and the working out of this law would require an extremely large number of measurements taken from individuals each one with its life history known," and again: "While it is probable that even this chance is according to fixed law, the fact remains that in any event this law is beyond a possibility of formulation from any observations except those extending over far more individuals than those here used." If as we believe the particular size variation of any individual bee depends on the size of the cell in which it grows, then the formulation of this law of variation must be

based on the law which governs the queen in moving over the comb and in choosing where she shall lay her eggs and on the law which governs the bees in cell building. We leave it to our critic to formulate these laws since we confess to a lack of mathematical ability for any such problems.

Finally we acknowledge the mistake in averages mentioned by Mr. Lutz but can only state that we believe this to be the only correct criticism of our paper which he has made.

Zoölogical Laboratory,
University of Pennsylvania.

NOTES ON A PECULIAR ACTINOZOAN LARVA.

L. R. CARY.

For a number of years past a few specimens of a large trochophore-like larva have been taken each summer in the tow near Beaufort, N. C., but they have never been seen to transform.

While at the laboratory of the United States Fish Commission at Beaufort,[1] during the past summer, I had the good fortune to secure a number of these peculiar larvæ.

The larvæ were taken while towing outside the harbor on August 15, after a heavy southeast storm which had continued for two days, and which had driven in shore specimens of several forms not usually found inside the Gulf Stream.

These larvæ were taken to the laboratory and at the suggestion of Dr. Caswell Grave, of the Johns Hopkins University, were put in aquarium jars of sea-water containing sand rich in diatoms. By this method they were kept alive for the remaining seven weeks of my stay at the laboratory.

The larvæ, Figs. 1 and 2, are elongate-oval in shape when in an active state, changing to a very nearly spherical form when they are disturbed. They are from two to four millimeters in length, and of a light brown or cream color.

At a point about one fourth of the distance from its anterior end, the body is encircled by a ridge which lies at the bottom of a shallow groove. This ridge bears on its surface two parallel bands of long stiff setæ, Fig. 4. The setæ-bearing ridge is not continuous around the body, but on one side it is interrupted and the ends overlap for a short distance, Fig. 1.

The whole surface of the body is provided with a covering of short cilia which are the true locomotor organs. The function of the bands of setæ is not apparent. They move only at irregular intervals, and then the force of their movement is in a direction antagonistic to the progress of the larva.

[1] I am indebted to the Hon. G. M. Bowers, U. S. Commissioner of Fisheries, for the privilege of occupying a table at the Beaufort Laboratory, and to Dr. Grave, the director, for many favors received while there.

At both the anterior and posterior ends of the body there is a depression lined with cilia. The depression at the anterior end has at its bottom an opening communicating with the interior.

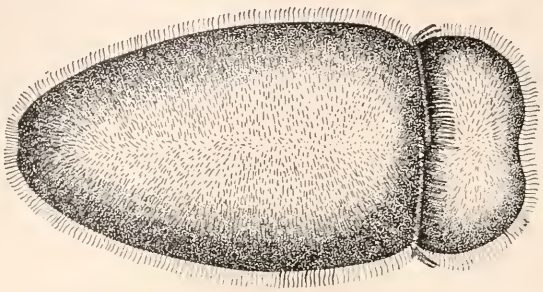


FIG. 1. Larva in the active expanded condition, × 30.

In swimming, the larva rotates rapidly on its long axis, and the anterior end describes a small circle about the axis of progression so that the larva advances by a kind of corkscrew

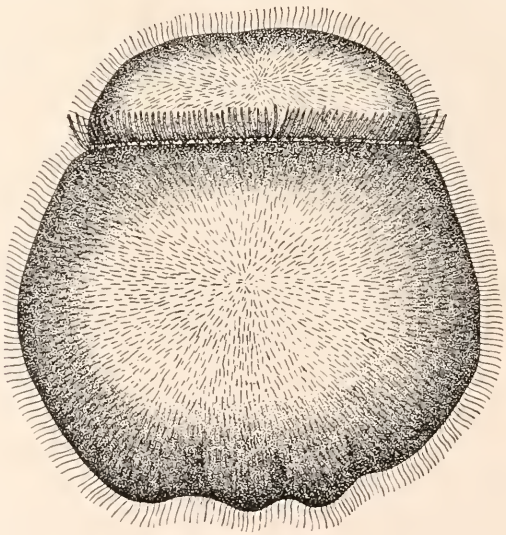


FIG. 2. Same larva as in Fig. 1, when contracted, × 45.

movement, such as has been described for the larva of *Astroides* by Lacaze Duthiers[1] and for *Renilla* by E. B. Wilson.[2]

[1] Lacaze Duthiers, H., "De Devellopment des coralliaires," *Archiv Zool. exper. et gen.*, Tom II., 1873.

[2] Wilson, E. B., "The Development of *Renilla*," *Phil. Trans. Roy. Soc. London*, Vol. CLXXIII., 1883.

The habits of the larva differ from those of the larvæ just mentioned in that it does not come to the surface of the water in the aquarium, nor does it remain motionless for any appreciable time unless it is disturbed, when it contracts and sinks to the bottom of the vessel.

Two of the larvæ went through their transformation two days after they were brought into the laboratory. When transformation is to take place, the larva settles down and becomes attached by the anterior end. In a short time the tentacles are budded out from the upper (posterior) end, and the mouth opening

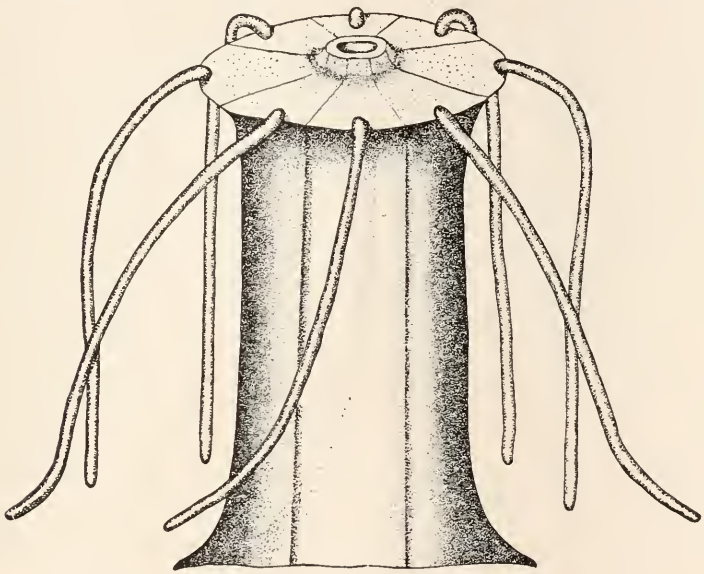


FIG. 3. Young polyp, two days after transformation, × 60.

breaks through the body wall within the circlet of tentacles. Within twenty-four hours from the time of the attachment at the beginning of the transformation, the young polyp had assumed a form such as is shown in Fig. 3.

In the five or six weeks after transformation, during which the young polyps were under observation, there was no apparent external change other than a gradual increase in the size of the animal as a whole without any change in proportions.

All the larvæ, with one exception, had transformed by Sep-

tember 7, three weeks after they were secured. The other specimen had not transformed on October 3, and, as far as could be determined, it had undergone no changes in size or form.



FIG. 4. Diagram to show position of setæ.

The actinian, of which the larva just described is the immature form, is not definitely known to me at present, but a number of mature sea anemonies, probably of the genus *Amophyllactis*, were cast upon the beach near where the larvæ were found during the same and subsequent storms coming from the same direction. Since both these forms appear only after heavy storms from a definite direction, and since there are certain structural resemblances between the young polyps and the mature actinians, it seems not improbable that they may be different stages in the life history of the same species.

ZOOLOGICAL LABORATORY,
JOHNS HOPKINS UNIVERSITY,
March, 1904.

BIOLOGICAL BULLETIN

THE MORPHOLOGY OF THE MADREPORARIA. V. SEPTAL SEQUENCE.[1]

J. E. DUERDEN.

The skeleton of an ordinary polycyclic hexameral coral presents a series of septal partitions arranged in a radiating manner, and in any cycle the constituent septa are equal in size and alternate in a regular manner with the members of the other cycles. In general, the cyclic plan of a corallite is 6, 6, 12, 24, 48, etc., and the number of septa in any cycle beyond the first corresponds with the total number in all the cycles within. As the septa in any cycle are alike in size it seems reasonable to suppose that they all appeared simultaneously in the growth of a coral, a cycle at a time, and also that the inner larger cycles were developed before the outer smaller cycles ; in other words, the relative sizes and positions of the septa in the mature corallite would seem to represent their order of development.

Much attention has already been given to the subject of the order of appearance of the septa in corals by different writers. The rule which Milne-Edwards and Haime give in their "Histoire Naturelle des Coralliaires" (1857) is well known, appearing in all text-books describing recent or fossil corals. The authors assume that in the case of the first three cycles the constituent septa of each cycle appear simultaneously, a cycle at a time, and that the relative development of each cycle corresponds with its relative size or distance from the center of the calice. From the fourth cycle outwards, however, a different sequence is

[1] The first two parts of this series of papers appeared in the *Johns Hopkins University Circulars*, Vol. XXI., Nos. 155 and 157, and were reprinted in the *Annals and Magazine of Natural History*, Ser. 7, Vol. X., May and August, 1902. The third and fourth parts appeared in the *Annals and Magazine of Natural History*, Vol. X., November, 1902, and Vol. XI., February, 1903. The work is being carried out with the assistance of an appropriation from the Carnegie Institution.

Contributions from the Zoölogical Laboratory of the University of Michigan. No. 81.

assumed for the various members of a cycle, according to their relation to the members of the inner cycles.

Professor A. Schneider in 1871 and Professor C. Semper in 1872 also discussed the same subject. The former agrees with Milne-Edwards and Haime as regards the manner of development of the first three cycles: first a cycle of six appears, then a smaller cycle of other six alternating with the first, and later a cycle of twelve septa which are smaller and alternate with the twelve making up the first and second cycles. In the further growth Schneider considers that the septa of a newer cycle may so enlarge in size as to appear to belong to an older cycle, and thus the primary sequence and hexamerism become lost.

Semper holds that no constant rule for septal development can be established, that the manner of growth varies more or less with each species.

Professor G. von Koch, in the course of his prolonged studies on the morphology of corals, has also made certain observations upon the laws of septal development, particularly in his paper "Das Vermehrungsgesetz der Septen," 1881. His results are based mainly upon serial sections of individual coralla of *Caryophyllia*, and lead him to conclude (p. 93) in the main in favor of the validity of the sequence given by Milne-Edwards and by Schneider: "Bei den sechszahligen Korallen, sowohl den Eporosen als den Perforaten, wachst die Zahl der Sternleisten (Septa) in der Art, dass sich nahezu gleichzeitig im ganzen Umfang des Kelches zwischen je zwei alteren eine jungere anlegt, also die Zahl der Sternleisten eines folgenden Cyclus immer gleich ist der Summe aller vorher vorhandenen. Alle Ausnahmen von dieser Regel sind auf direxte Anpassungen oder erblich gewordene Veranderungen im Wachsthum des ganzen Thieres zuruckzufuhren."

All the above conclusions are based mainly upon the examination of adult coralla, in which there is available for comparison only the relative sizes of the septa and their order of appearancein serial sections. The actual details of growth of the septa indeveloping corals, in their relationships to the mesenteries, have in no instance been followed beyond the first two cycles. Professor H. de Lacaze-Duthiers (1873, '94, '97) and Professor G. von Koch (1882, '97) have both studied the early development of the skeleton in

corals, but their results throw no certain light upon the difficult problem of the manner of increase of the septa beyond the two primary cycles, nor of the relation of these to the later cycles.

Observations which I have been able to make upon the growth of the septa in larval polyps of the common West Indian coral, *Siderastrea radians* (Pallas), show the unreliability of assuming the developmental sequence from adult relationship, and give an interpretation to the later septal sequence altogether different from any hitherto proposed.

Larvæ of *Siderastrea*, fixed to fragments of glass, and capable of being examined as transparent microscopic objects, were followed in the course of their development as young polyps for a period of four months, and the order of appearance of their septa determined as far as the completion of the first three cycles.

In every case it was found that the six members composing the first cycle appeared simultaneously. This takes place shortly after the larva settles, at a stage when the young polyp has six pairs of mesenteries, arranged as in Fig. 1, where all the mesenteries are complete except the fifth and sixth developmental pairs. The septa are alike in size and situated at equal distances apart within the entocœles of the six primary pairs of mesenteries. Later development proves, as would be expected, that the six primary septa of the mature corallite are the direct enlarged representatives of the six septa first to appear in the larval polyp.

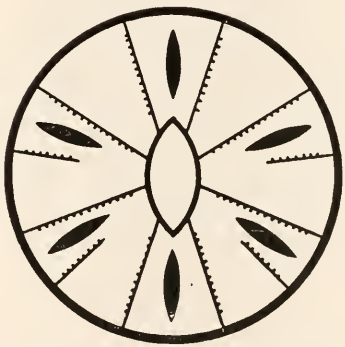


FIG. 1. Diagrammatic arrangement of the mesenteries and septa on the appearance of the first cycle of six septa. The septa are situated within the six primary entocœles, and the six alternating mesenterial chambers, devoid of septa, are the primary exocœles. The directives are situated at the opposite extremities; the two bilateral pairs of incomplete mesenteries are the fifth and sixth pairs in the mesenterial sequence. The upper border is regarded as dorsal and the lower as ventral.

As regards the primary cycle in *Siderastrea* the surmise that adult size corresponds with developmental sequence is therefore correct. Moreover, in the early

stages of septal growth secured by Lacaze-Duthiers in *Astroides*, *Flabellum*, *Balanophyllia*, *Caryophyllia*, etc., and also by von Koch in *Astroides* and *Caryophyllia*, the six primary septa appeared simultaneously, and were equal from the beginning.

In *Siderastrea* the second cycle of six septa began to appear a few days after the primary cycle, its members situated within the exocœlic chambers, thus alternating with the six entosepta (Fig. 2). In a few polyps the septa appeared simultaneously and were all practically equal, but in most individuals a marked difference was manifest; the dorsal and middle pairs of exosepta arose bilaterally in advance of the two ventral pairs, and for a time the dorsal pairs were a little larger than the middle. Within a short time the two ventral exosepta appeared, but remained smaller than the others. Thus at this early stage a decided dorso-ventrality in the development of the septa was apparent, which gave a bilateral character to the polyp as a whole (Fig. 2).



FIG. 2. Arrangement of the mesenteries and septa after the establishment of the first and second septal cycles — six entosepta and six exosepta. The dorsal pair of exosepta are somewhat larger than the middle pair, and the middle pair are larger than the ventral pair, thus giving a bilateral character to the corallum.

In most of the developing corals investigated by Lacaze-Duthiers and von Koch the entocœlic and exocœlic cycles either appeared together, or one shortly after the other, as in *Siderastrea*. In most cases it was found that the members of either cycle arose simultaneously, in a truly radiate manner, without passing through a bilateral stage, though Lacaze-Duthiers figures a very decided bilateral condition in the early development of the skeleton in *Astroides* (1873, Pl. XIII., Fig. 29).

The next stage in the growth of the septa in *Siderastrea* is well defined, but is not so clear as to its significance. After the condition shown in Fig. 2 was reached the septa began to enlarge at their peripheral extremity; in some instances the enlargement

took place by the direct extension of the septa already developed, and in others by the deposition of separate skeletal nodules. The new growth was arranged in a V-shaped manner, the angle of the V being larger in the exosepta than in the entosepta (Fig. 3). In the diagrammatic figure the additions are all represented as separate calcareous fragments, but no constancy was apparent in the different septa as to the freedom or fusion of the extensions at this stage. In all the polyps the enlargement of the ventral exosepta was much behind that of the dorsal and middle exosepta, and usually it could be seen that the middle exosepta did not enlarge as rapidly as the dorsal.

At a somewhat later stage, each septum became a continuous structure, owing to the complete fusion of the free nodules in the

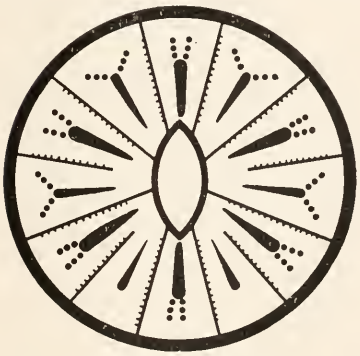


FIG. 3. Stage showing the peripheral enlargement of the septa which occurs in a bifurcating manner by the addition of separate nodules. The ventral exosepta grow more slowly than the middle and dorsal pairs.



FIG. 4. Further enlargement of the septa by fusion of the nodules, and establishment of the six pairs of second cycle mesenteries ; the latter, like the exosepta, decrease in size from the dorsal to the ventral border.

process of growth. The entosepta then appeared as simple formations, much broader peripherally than centrally, though the two directive entosepta showed traces of the earlier bifurcation longer than the four lateral entosepta ; the exosepta, on the other hand, remained strongly bifurcated, with the exception of the ventral pair, the members of which enlarged comparatively little (Fig. 4).

A similar bifurcated stage, due to the appearance of independent calcareous nodules, occurs in the development of *Astroides*,

Caryophyllia, and some other corals, but hitherto its significance has not been satisfactorily explained. At one time the separate fragments were supposed to be concerned in the formation of the thecal wall, but it will be seen that such is certainly not their fate in *Siderastrea*.

About this time, the polyps being two months old, the second cycle mesenteries began to appear on the column wall, situated in the exocœlic chambers between the primary mesenteries (Fig. 4). In their development they also presented a conspicuous dorso-ventrality : the two first pairs appeared within the dorsal exocœles, the moieties of each pair arising at the same time and remaining equal ; the two next pairs were within the middle exocœles ; and finally appeared the pairs within the ventral exocœles. The dorso-ventral succession thus followed by the six pairs remained evident throughout the period under observation, the dorsal pairs being larger than the middle, and the middle larger than the ventral. The comparative development of the first and second cycle mesenteries at this period, and their relationship to the septa, are shown in Fig. 4. A similar bilateral, dorso-ventral succession of the second cycle mesenteries has long been known to occur in the development of actinian polyps, in contrast to the simultaneous origin at one time assumed.

Clearly the next skeletal stage will be one involving the establishment of septa within the entocœles of the second cycle of mesenteries, and these will constitute the second cycle of septa of the adult corallite. Hitherto it has been generally assumed that where a cycle of exosepta is already developed, and then a new cycle of mesenteries appears within the corresponding exocœles, that the exosepta already present become included within the entocœles of the new mesenteries, and thus become entosepta ; an additional outer cycle of exosepta appears later and its members in their turn become entosepta. Thus Delage and Hérouard in their " Traité de Zoologie Concrète " (1901, p. 558) remark : " Quand, dans les interloges occupées par les septes du dernier cycle, naît un nouveau cycle de couples de cloisons, celles-ci se forment de part et d'autre du septe interloculaire qui, de ce fait, devient loculaire, et bientôt un nouveau cycle de septes se forme dans les nouvelles interloges qui vien-

nent d'être formées. Les cycles naissent successivement et jamais un cycle ne commence à se former avant que le précédent soit complete." Similarly J. Stanley Gardiner (1902, p. 133) in his account of the anatomy of *Flabellum rubrum* says (italics added): "As the growth of any corallite proceeds, more and more septa up to six cycles appear. *The former exocœlic order of septa becomes entocœlic by the development of new pairs of mesenteries.* The increase of mesenteries takes place *pari passu* with the formation of new septa."

Unfortunately, the relationships involved in the above assertions have not been actually followed, though from the known conditions no other arrangement at first sight seems possible; and it was principally with a view to determine the truth or otherwise of the assumption that the present investigation was undertaken.

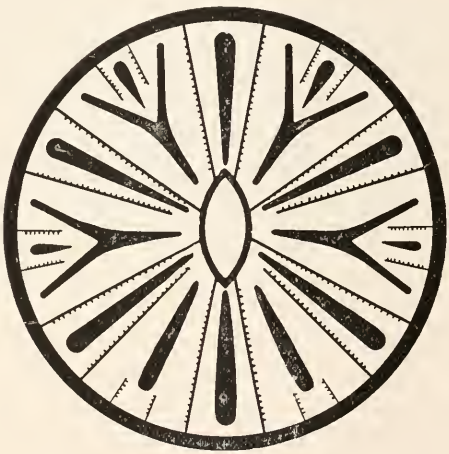


FIG. 5. First appearance of the permanent second cycle of entosepta situated within the entocœles of the second cycle of mesenteries and the bifurcations of the dorsal and middle exosepta.

Shortly after the stage represented in Fig. 4 was reached independent calcareous growths began to arise peripherally, in positions corresponding with the entocœles of the second cycle mesenteries; those within the dorsal entocœles were larger than the ones within the middle entocœles, while for a long period there was no corresponding formation within the ventral entocœle (Fig. 5). At first the structures were quite free and resembled

small independent septa ; they suggest a new series of entocœlic septa, appearing in a dorso-ventral sequence like the mesenteries with which they are associated.

Later these new septa extended more centrally, and necessarily came into union with the simple inner portions of the septa which originally constituted the exocœlic second cycle. Several of the polyps were reared until the new second and third orders of septa were fully established, when they presented the arrangement shown in Fig. 6. The peripheral, primarily independent septa

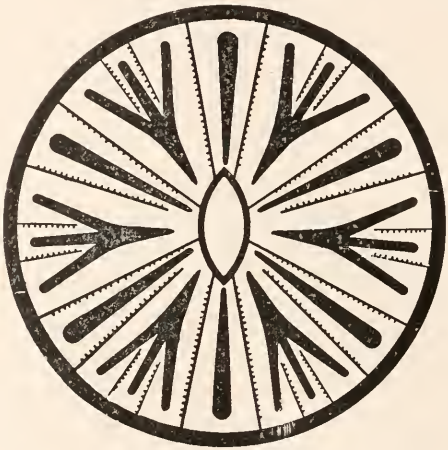


Fig. 6. Completion of the first three cycles of septa.

within the second cycle entocœles have all become continuous with the median part of the original second cycle exosepta, and along with them now constitute the permanent second cycle of entosepta ; while the bifurcations of the exosepta now constitute the third cycle of twelve septa and are seen to be exosepta.[1] It will also be seen that the growth within the ventral system has now attained the same stage as that within the middle and dorsal systems, so that the corallite as a whole presents nearly perfect radial symmetry.

The stages thus passed through are of great importance in their bearing upon several obscure points in coral development and morphology, and call for fuller consideration. In the first

[1] In the adult corallite of *Siderastrea radians* the exosepta are fused by their inner ends with the entosepta as represented in Fig. 6 and Fig. 7*s*.

place, it is seen that the second cycle entosepta, which are to become the permanent second cycle of the adult corallite, arise as independent formations, though later they fuse with the septa which constituted the original second cycle, the two series being situated along the same radii. They are to be regarded as altogether new formations which replace the original second cycle exosepta; the fact that they fuse with the latter in their forward growth would seem to be of incidental importance, depending upon the fact that they are in the same radii. Secondly, the original second cycle exosepta of Fig. 2 lose their morphological individuality, becoming involved in the new second cycle entosepta as the latter continue their growth centrally; they are merely the temporary predecessors of a later permanent cycle.[1] As primary second cycle exosepta they do not become included within the entocœles of the second cycle mesenteries, but only as the central continuations of the new second cycle entosepta.

The results thus afford definite proof that the exosepta of a former stage do not become the entosepta of a later stage when another series of mesenteries has appeared with the entocœles of which they correspond. It is manifest that such a conclusion could only be established in *Siderastrea* by actual observation of all the intermediate stages. When the condition represented in Fig. 6 has been reached there is no means of determining the actual two-fold origin of the second cycle entosepta. It is such conditions which have hitherto been studied, and from these no other explanation than that given by Delage and Hérouard and by Gardiner on page 70 would have been expected. Entosepta throughout would now appear to be new formations, not the continuations of the exosepta of a previous stage, and further, they arise after the mesenteries within the entocœles of which they are situated.

[1] In many corals the original second cycle exosepta appear to continue their independent growth *in situ* without losing their identity in the central extension of the entosepta. I believe it will be found that this is the true nature of *pali*, which are found in some corals as small septum-like plates in front of the larger septa. The fact that pali seem not to occur before the primary cycle of six septa, but only before those of later origin, is what we should expect if this surmise be correct. The primary entosepta have never had exocœlic predecessors, as is the case with the later entosepta. Pali would thus represent the persistent exocœlic predecessors of the entosepta beyond the primary cycle which have not lost their individuality in the later growth of the entosepta.

The significance of the twelve exosepta which constitute the outermost third cycle remains to be considered. They undoubtedly represent the direct continuations of the bifurcations of the six primary second cycle exosepta, but their relationships have now changed; instead of appearing as extensions of an older cycle of septa they themselves constitute a cycle. The details exhibited by the particular species studied seem inconclusive towards determining whether the exosepta of the later stage are to be regarded as but continuations of the exosepta of the previous stage or as entirely new formations. The former would certainly seem to be suggested; probably the point could be definitely established in some other coral species in which the exosepta in the adult are not fused at their inner extremity with the entosepta. Whichever view is accepted the exosepta of the third cycle are obviously developed in advance of the entosepta of the second cycle.[1] Thus the relative sizes and positions which the cycles will ultimately assume in the mature calice do not represent their actual order of appearance.

A third important morphological relationship is definitely established, namely, that the later septa do not arise a cycle at a time as is usually assumed, any more than do the mesenteries. Beyond the primary cycle of six entosepta, the members of which always appear simultaneously, there is a decided dorso-ventrality in the sequence of the septa of each cycle, which for the time being gives a marked bilateral symmetry to the corallum. It is only later, when the development of the septa within each sextant has reached the same stage, that an approximate equality and radial symmetry is attained. Thus the primary symmetry in corals is bilateral, and the arrangement is retained for a long period in the ontogeny of both mesenteries and septa. Therefore in the individual septa making up a cycle, as has been proved for the cycle itself, the adult size does not represent the actual order of appearance.

To return to the further development of the septa in *Sideras-*

[1] It is worthy of note in this connection that the exotentacles in *Siderastrea radians* have been found to appear thoughout in advance of the entotentacles, being the only zoantharian in which this relationship is known to occur. Hence there is nothing contrary to the laws of hexactinian development in the above conception that the exosepta beyond the first series appear in advance of the corresponding entosepta.

trea. The larval polyps were not reared beyond the completion of the first two cycles of mesenteries and the first three cycles of septa (two cycles of entosepta and one of exosepta). On any colony, however, are many developing polyps which present intermediate stages between the commencement and completion of the third cycle of mesenteries and the fourth cycle of septa; and from these results have been obtained supplementary to those already presented. Mature polyps of *S. radians* have rarely more than three cycles of mesenteries and four cycles of septa (three inner cycles of entosepta and an outermost cycle of exosepta).

First, it may be ascertained what are the relationships between the third cycle exosepta of Fig. 6 and the third cycle entosepta and fourth cycle exosepta found in the mature corallite. By means of serial sections through decalcified immature bud polyps it has been possible to establish the relationship of these with respect to one another and to the new mesenteries of the third cycle. The results are diagrammatically represented in Fig. 7 (*a* — *g*), the complications due to the presence of synapticula being omitted. Fig. *a* represents a section through an exoseptum of the third cycle, corresponding to one of the exosepta in Fig. 6, only that the peripheral extremity is now bifurcated. It is in the same stage as each of the four exosepta of the second cycle in Fig. 4, but is shown united with the calicinal wall, and no mesenteries have as yet appeared within the two limbs. Fig. *b* shows the same septum taken from a section at a higher level. Within the exocœlic bifurcation there has now appeared a pair of third cycle mesenteries, in every way comparable in their relationships to the third cycle exosepta with those of the second cycle mesenteries to the second cycle exosepta of Fig. 4.

Fig. *c*, from a still higher level of the polyp, reveals a further stage. Within the entocœle of the mesenteries is seen the rudiment of a third cycle septum. The latter is a new formation, comparable with the rudiments of the second cycle entosepta shown in Fig. 5. One limb of the exoseptum has also become free.

Fig. *d*, taken from a section above that of Fig. *c*, shows the new entoseptum becoming larger, and extending centrally further

than the mesenteries, which in their turn have also increased in size. Both limbs of the bifurcated septum are now free from the middle portion ; they represent two independent exosepta becoming distinct from the single exoseptum of a previous cycle (*cf.* Fig. 6) of which they were originally continuations.

Fig. *e* is from a section through the column wall, the septa at this level being exsert, that is, extending above the calicinal wall.

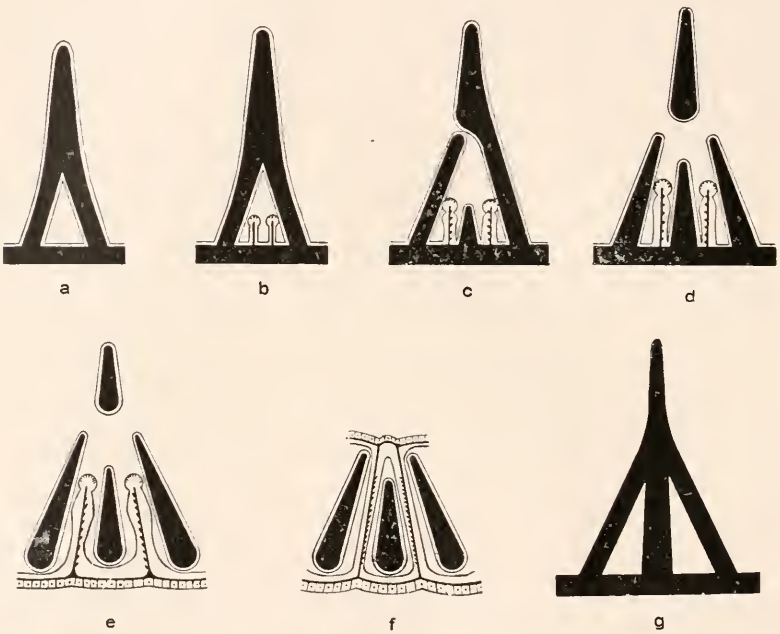


FIG. 7 (*a–g*). Series of diagrammatic figures illustrating the developmental relationships of a pair of third cycle mesenteries and a third cycle entoseptum, in association with a bifurcated third cycle exoseptum.

The relationships shown are the same as in the previous figure, but the inner radial portion of the original third cycle exoseptum has almost disappeared. Fig. *f* is through the column wall (lower) and disc (upper). The mesenteries now extend from one wall to the other, the polyp being in the retracted condition, and the entoseptum is still smaller than the exosepta, one on each side of it. It is manifest that in the later growth the entoseptum will extend more centrally, and come into union with the original third cycle exoseptum which is in the same radius (Fig. *g*), exactly as in the larval polyp shown in Figs. 5 and 6.

The series of sections thus reveals that the third and fourth cycles of septa and the third cycle of mesenteries are related in the same manner as are the second and third cycles of septa and the second cycle of mesenteries; the third cycle entosepta have third cycle exocœlic antecedents. The results may be arranged as follows:

(*a*) The originally simple third cycle exosepta, themselves formed as bifurcations of simple second cycle exosepta, become bifurcated at their peripheral extremity.

(*b*) Within each bifurcation there appears a new pair of third cycle mesenteries, and then within the entocœle of the pair is formed a third cycle entoseptum.

(*c*) The new entoseptum fuses with the central portion of the third cycle exoseptum, while the bifurcations of the latter constitute two new exosepta of the fourth cycle, and are fused at their inner extremity with the entoseptum embraced by them.

Presumably the same process as above outlined will be followed within the two exocœlic chambers of each sextant of the polyp, so that in the end there will be twelve entosepta forming the completed new third cycle and twenty-four exosepta forming the completed new fourth cycle.

The actual sequence according to which the third and fourth cycles of septa are formed remains to be noticed.

The septa alone in the dried corallum are insufficient for this purpose, as they afford no certain means by which the directive axis can be determined, and from this the dorsal and ventral borders of the calice. It has been shown, however, that the mesenteries associated with the entosepta appear in pairs only a little in advance of the entosepta within them; therefore if the sequence of the mesenteries be determined it can be assumed that the septa follow the same order.

From colonies of *Siderastrea* sections of a large number of bud polyps at different stages of development have been prepared, and from these it has been possible to determine the order of appearance of the twelve pairs of third cycle mesenteries. This is indicated in the series of diagrammatic figures in Fig. 8 (*a*–*d*). In Fig. 8 *a*, in addition to the primary and secondary cycles, a pair of third cycle mesenteries (III.) has appeared on each side of the median axis, situated in the exocœle between

the dorsal directives and the dorsal pair of second cycle mesenteries. Such an early stage is to be expected on the dorso-ven-

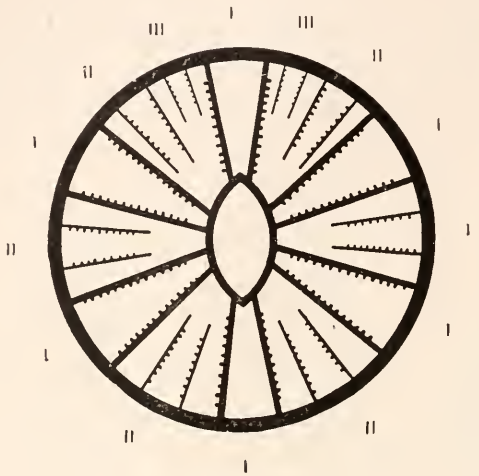


FIG. 8*a*.

FIG. 8 (*a*–*d*). Series of diagrammatic figures illustrating the order of development of the twelve pairs of third cycle mesenteries.

tral succession already established in the case of the second cycle mesenteries (p. 70).

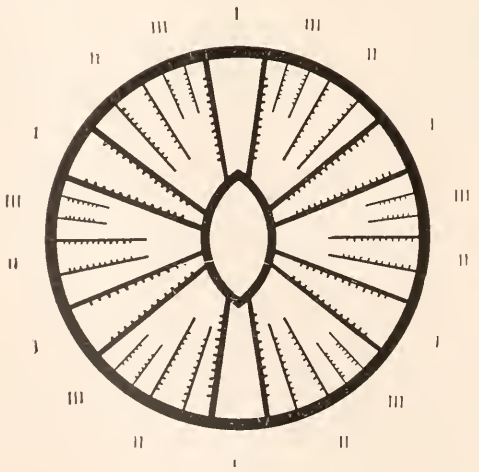


FIG. 8*b*.

The succeeding exocœlic chamber on each side lies between the dorsal pair of second cycle mesenteries and the dorso-lateral

pair of first cycle mesenteries, and it might be supposed that the new mesenteries would occupy the exocœles in regular succession from one aspect of the polyp to the other. Instead of this

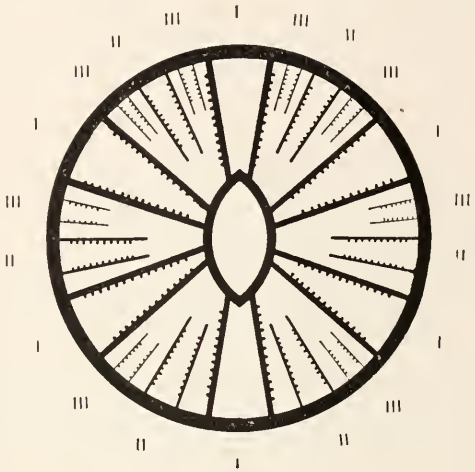


FIG. 8*c*.

the pairs are found to arise successively within only the dorsal member of the two exocœles of each system. This is shown

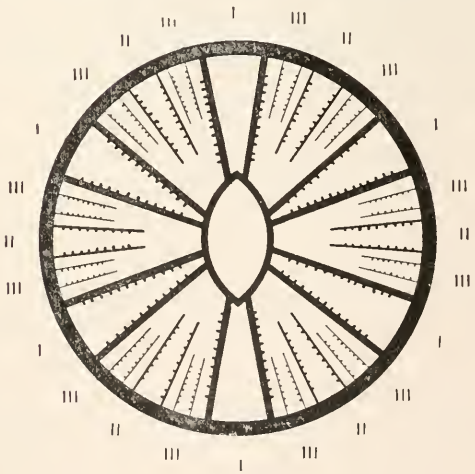


FIG. 8*d*.

in the next stage available, Fig. 8*b*, where a third cycle pair is found within the dorsal exocœle of each sextant.

A further stage secured in the growth of the twelve pairs of third cycle mesenteries is given in Fig. 8*c*, where a pair has appeared on each side within the ventral exocœle of the dorsal sextant. Clearly, if the succession thus indicated were followed with perfect regularity, other pairs would appear within the ventral exocœles of both the middle and the ventral sextants, and the cycle would then be completed according to Fig. 8*d*. No stage exactly corresponding with Fig. 8*d*, however, has been obtained, as the polyps of *S. radians* very rarely, if ever, complete the third cycle of mesenteries. Still the sequence so far as it can be traced is such as to warrant the conclusion.

The regularity in the dorso-ventral sequence of the mesenteries shown in Fig. 8 was secured only after an examination of a number of polyps. In a colony in which the polyps are so closely arranged as in *S. radians* the individuals are rarely found to undergo their later development with perfect regularity all round; some regions will be in advance of the normal sequence and others behind. The polygonal form assumed by the adults is evidence that a certain pressure is exerted upon a form which would otherwise be circular, as in the simple polyps reared from larvæ. Spatial difficulties may therefore be held sufficient to account for the irregularities appearing in the growth of the third mesenterial cycle. In *Astrangia solitaria* and *Phyllangia Americana*, where the polyps are practically free from one another, and retain their cylindrical form throughout, the regularity of development from one border to the other is more pronounced, and I have found (1902, p. 459) the order of appearance of the mesenteries to be the same as that established for *S. radians*.

The normal sequence of the third cycle mesenteries in *Siderastrea* being now established we are justified in assuming that a like succession will be maintained by the third cycle septa, as individually the septa arise shortly after the mesenteries with which they are associated. Hence the normal sequence for the members of the first three cycles of entosepta will be that represented in Fig. 9. The six septa of the first order of entosepta (I.) appear together as a cycle; the six members of the second (II.*a*–II.*c*) follow a simple dorso-ventral succession; the twelve members of the third order (III.*a*–III.*f*) also appear in a dorso-

ventral succession, but in two series — first a series of six (III.*a*–III.*c*) within the dorsal of the two interspaces in each sextant, and then the remaining six (III.*d*–III.*f*) in a like order but within the ventral of the two interspaces. As explained below, the exosepta (X.) constituting the last outermost cycle have not the same ordinal significance as the entosepta.

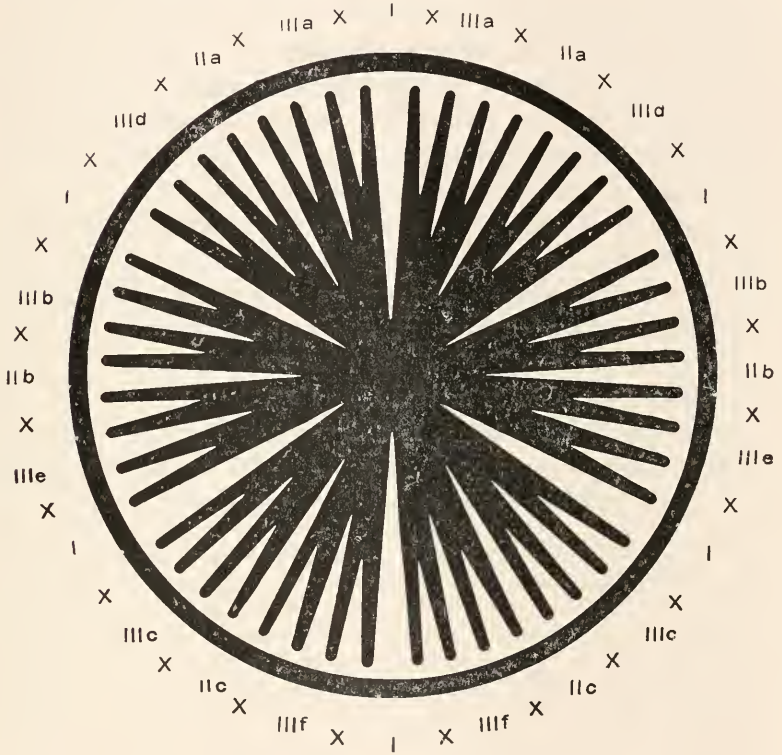


FIG. 9. Diagram showing the order of appearance of the septa in a corallite with three cycles of entosepta (I.–III.) and an outer cycle of exosepta (X.). The Roman numerals indicate the cycle to which the septa belong and the letters their sequence in the cycle.

Studies on the mesenterial sequence of other corals indicate that a similar septal succession will in all probability be followed by most forms in which the adult calice shows a regular hexameral cyclic plan. Individual departures from the order may be expected, but are to be looked upon as irregularities; regularity of growth of the higher cycles of mesenteries and septa is

by no means so pronounced as in the first and second cycles which are less likely to be influenced by spatial considerations. The sequence given is altogether different from anything which has hitherto been surmised for any coral, and further studies are desirable to determine how far it admits of general application in the group.

From what has already been revealed it is manifest that the exosepta of corals do not possess any true ordinal sequence comparable with that of the entosepta. Exosepta have been found to be present at each developmental stage, always constituting the outermost cycle, and equalling in number the sum of the inner entosepta ; but until the adult condition is reached they are merely the predecessors of the entosepta. We may consider them as the direct continuations of the primary six exosepta which bifurcate at each stage, or, less likely, as arising anew with each cycle of entosepta. Regarded as the persistent representatives of the primary exosepta they more nearly conform to the "law of substitution" in actinian tentacles as established by Lacaze-Duthiers (1872) and Faurot (1895).[1]

Studies on other corals, as well as considerations on the tentacular development in actinians, suggest that the exosepta may arise in different ways in different species of corals, and that a more

[1] In actinians generally it is found that after the protocnemic stage the tentacles appear two at a time, one entocœlic and one exocœlic, corresponding with the two chambers formed upon the appearance of a new pair of mesenteries; sometimes the entotentacles appear in advance of the exotentacles, though in *Siderastrea radians* the reverse is the case. The entotentacles when established are larger than the exotentacles, the length of the former being in accordance with the order of appearance of the cycle to which they belong, the largest being the first to appear. The exotentacles all attain an equal length and throughout are relegated to the outermost cycle, whatever be the cycle of entotentacles with which they first appeared. They constitute a single cycle of which the members are smaller than those of the cycle of entotentacles last to appear. The number of exotentacles in the last cycle is always half the total number of tentacles, the number of exocœles being equal to that of the entocœles.

Being soft polypal structures it is easy to understand how as new entotentacles are added the exotentacles become pushed aside and thus occupy different radii at different times. The septa, being hard fixed structures, do not admit of rearrangement; the new growth has to be adapted to the old, as in the fusion of the new entosepta with the old exosepta.

The tentacles, like the septa, thus arise in such a manner that it is impossible to determine their order of development from their relationships in the mature polyp.

precise significance as to their relationships at different stages may be forthcoming than is possible in *Siderastrea*. There are indications that in some forms an entoseptum and an exoseptum arise together, thus more closely recalling the method followed in the appearance of the tentacles.

The relationships now proved to exist between the entosepta and exosepta of corals involve important considerations when the cyclic hexameral sequence is not completed in the mature corallite, a condition which almost invariably happens in *S. radians*, as well as in numerous other corals. As regards both septa and mesenteries it is found in such cases that the last cycle is rarely a multiple of six, but some irregular number, resulting from the fact that at maturity the polyp does not complete the last cycle begun. Exosepta have been shown to appear always in association with entosepta, whatever be the number making up a corallite, and, as often remarked, the two series are equal in number and the exosepta outermost in position. Hence it follows that in the cyclic incompletion of the mature corallites of *Siderastrea* the third entocœlic cycle and fourth exocœlic cycle of septa vary in the same degree; whatever number of entosepta be lacking to form the complete third cycle of twelve a like number will be wanting from the twenty-four exosepta which should form the fourth cycle.

When describing the number of septal cycles within a calice of which the cyclic hexameral plan is incomplete it is usual in systematic works on corals to regard the hexameral multiples as completed so far as the number of septa will permit, and then to relegate to the last cycle all the surplus septa not included in the hexameral formula. The cycles are all supposed to be hexamerously complete with the exception of the last. Thus, with regard to *S. radians*, Milne-Edwards states: "Three cycles of septa complete, and, in general, a variable number of a fourth cycle"; likewise Verrill (1901, p. 133), describing the same species, says: "They [the septa] form three complete cycles, with part of the fourth cycle developed, so that the number is usually 36 to 40."

The relationships now established between the entosepta and

exosepta indicate that the above formulæ do not express the true morphological character of the septa. Any hexameral incompletion in the number of septa making up a corallite affects both the entosepta and the exosepta, that is, both the penultimate and the last cycles ; if any septa be wanting to complete the hexameral multiple of the last cycle of entosepta the same number will be wanting from the outermost cycle made up of exosepta. The third complete cycle as understood by Milne-Edwards and by Verrill is really made up of both tertiary entosepta and of tertiary exosepta. The two kinds of septa are obviously of very different value in their development and relations to the mesenteries, and, as a matter of fact, will scarcely be of the same thickness and radial length to justify their being regarded as members of one cycle.

The cyclic formula, as usually understood in systematic works, may be written, 6, 6, 12, X, where X will represent any number from one to twenty-four. Formulated in this way the number 12 conveys the impression that the third cycle is really completed, that all the remaining septa belong to the next or fourth cycle, and that it alone is numerically incomplete. But beyond the two first cycles the septa of the penultimate and last cycles are formed concurrently, or almost so, in pairs, and incomplete cyclic hexamerism, as met with in *S. radians*, is really an intermediate condition in the establishment of two adult hexameral cycles, not of one alone, and attention should be drawn to this in the septal formula.

According to the relationships above established the morphological septal formula for *S. radians* should be written 6, 6, X, $6 + 6 + X$. In this formula the numbers 6, 6, represent the septa in the first and second completed entocycles, and X the number in the third entoseptal or penultimate cycle which does not yet complete the hexameral sequence ; while $6 + 6 + X$ will represent the total number of exosepta, X being the same number as before. In the calice some of the exosepta will be tertiaries and some will be quaternaries, the number of the latter being always double the number of tertiary entosepta. The formula for a corallite having 36 septa would, according to the ordinary cyclic formula, be written, 6, 6, 12, 12, whereas, con-

sidered as entosepta and exosepta, the formula would be 6, 6, 6, 18; the three first numerals in the latter indicate the entosepta and the last the exosepta. The cyclic formula of a corallite with 40 septa would be 6, 6, 12, 16, and the morphological formula 6, 6, 8, 20. In the first morphological formula 12 of the exosepta will be quaternaries and 6 will be tertiaries; in the second 16 will be quaternaries and 4 tertiaries.

Where the relationships of the septa to the mesenteries are clearly known the morphological formula will more nearly express the real value of the septa than the ordinary cyclic formula, the latter has little significance unless the hexameral sequence is fully completed. One is not justified in saying that a cycle is really complete unless all its constituents have the same morphological value, and this is not the case when some are entosepta and some are exosepta.

A few remarks may here be made concerning the dorso-ventral appearance of the organs in corals generally, and the consequent marked bilaterally of the calice for the time being.

In palæontological literature much has been made of the fact that the Palæozoic rugose corals (*Tetracoralla*) are bilaterally symmetrical, while most modern corals are radially symmetrical. The results here outlined prove however that modern corals are strongly bilateral in the course of their development, and that it is only when the septa are fully established that an approximate radiality is assumed. In like manner I have found that many rugose corals attain perfect or almost perfect radiality when maturity is reached, though the developmental stages are strongly bilateral. Radiality in the *Actinozoa*, as compared with bilaterality, seems to have a more ontogenetic than phylogenetic significance. Furthermore, beyond the six primary members the septa in the *Rugosa* are added in a manner altogether different from that in modern hexameral corals, hence the bilaterality of the one group has a different origin from that of the other. The subject of bilaterality in the *Rugosa* will be more fully discussed in a later paper.

In modern corals the bilaterality of the polyp during development may be looked upon as associated in turn with each cycle

individually. Any cycle of septa or mesenteries tends to attain its radial condition before the next cycle commences to form, when the additions take place in such a manner as to again confer bilaterality upon the polyp as a whole. Thus the first two cycles of septa become truly radial before an additional cycle commences, when the growth of this is continued in a bilateral manner; likewise the new second and third cycles assume their radial stage before the members of the fourth cycle made their appearance, these also proceeding from one border to the other. In like manner the first cycle mesenteries are nearly radial before those of the second cycle arise and introduce a conspicuous bilateral symmetry; on the second cycle mesenteries assuming the radial plan the third cycle members begin to appear, again in a bilateral manner.

The successive dorso-ventral growth followed by the constituent mesenteries and septa of each cycle also confers a certain individuality upon the cycle. The different cycles, arising independently, seem to represent so many distinct recurring phases of growth in the life of the polyp; they do not constitute a continuous addition from one border to the other, as is usual in permanently bilateral animals, particularly segmented forms. The members of a cycle appear in a dorso-ventral sequence and may retain their differences in size for a long time, but in the end they become equal and thereby confer radial symmetry upon the polyp. Then another cycle commences to form in somewhat the same bilateral dorso-ventral succession, displays for a time the consecutive origin of its members, and afterwards attains radiality.

The conception of recurring phases of growth in cyclic coral polyps is best realized when comparison is made with the mesenterial increase characteristic of the Ceriantheæ and Zoantheæ. In the former the mesenteries beyond the protocnemes always develop in a regular bilateral successive manner, from the dorsal (anterior, sulcar) to the ventral (posterior, asulcar) aspect, the oldest being dorsal or anterior and the youngest ventral or posterior, recalling more the method followed by segmented animals; there is in the Ceriantheæ never a reversal of growth to the anterior end, followed by a successive series to the other, such as occurs in ordinary hexactinians. Employing the term "band of

proliferation," introduced by van Beneden in 1897, we may say there is only one median band of proliferation in cerianthids, while in hexactinians there are many such bands occurring all round the polyp, the number increasing with age — at first six, then twelve, twenty-four, etc.

In the Zoantheæ also mesenterial development is always in the same succession after the protocnemic stage. The increase takes place within only two of the six primary exocœlic chambers, one on each side of the ventral directives; there are only two bands of proliferation or zones of growth. In this case, however, the order followed by the new mesenteries differs from that in hexactinians and cerianthids; it proceeds from the ventral (posterior, sulcar) to the dorsal (anterior, asulcar) aspect of the polyp, not from the dorsal to the ventral.

The bilateral development of the organs, from one border of the polyp to the other, in ordinary actinians and corals would seem to have no phylogenetic significance beyond the group of the cœlenterates; indeed, even here we appear to have as yet no definite understanding as to what its meaning may be. The approximate radial symmetry of adult cœlenterates is assumed from very diverse developmental conditions (*cf.*, hexactinians, zoanthids, cerianthids, and the tentacles and other cyclic organs in the Hydromedusæ and Scyphomedusæ). Whatever may be said in favor of the well-known view that the mesenterial arrangement in cerianthids suggests the metamerism of higher animals there is clearly no support for such a conception in the development of the organs in hexactinians. In this latter group we are concerned with a radial cyclic repetition of the organs, even though the members of each cyclic series arise in bilateral succession from one border to the other.

Summary.

1. In the coral *Siderastrea radians* the six members of the first cycle of septa appear simultaneously, shortly after fixation of the larva, situated within the entocœles of the first cycle of mesenteries.

2. Six members of a second cycle are developed within the primary exocœles shortly after the primary cycle of septa. They are the temporary predecessors of a later permanent cycle,

and arise either simultaneously or in bilateral pairs in a dorso-ventral order. Later, they become bifurcated peripherally, either by the direct extension of the original septum or by the production of separate fragments which subsequently fuse. The bifurcations also appear in a bilateral dorso-ventral order.

3. The six members of the permanent second cycle of entosepta arise within the entocœles of the second cycle mesenteries soon after these make their appearance. The two right and left dorsal septa appear first, then the two middle members, and, at a much later period, the two ventral, the series thus exhibiting a decided dorso-ventrality. In the end they become equal, and each fuses with the central part of the corresponding second cycle exoseptum previously developed, these exosepta thereby losing their individuality.

4. Twelve members of a temporary third cycle are situated within the exocœles between the primary and secondary pairs of mesenteries, and represent the bifurcated extensions of the six primary exosepta. The original second cycle exosepta thus become the third exocœlic cycle, their place having been taken by the new second cycle of entosepta.

5. A new third cycle of twelve (or less) septa arises on the appearance of the pairs of third cycle mesenteries, in a similar manner to that followed by the second permanent cycle. New entosepta appear within the entocœles of the third cycle mesenteries, and the bifurcations of the third cycle exosepta then become the exosepta of the fourth cycle.

6. The third cycle entosepta, following the mesenteries, are developed in a bilateral dorso-ventral order, but in two series; first a series within the dorsal moiety of each sextant, and then a second series within the ventral part of each sextant.

7. Exosepta are present at each cyclic stage in the growth of the corallum, alternating in position and corresponding in number with the sum of the entosepta. They never become entosepta, but always constitute the outermost cycle of shorter septa; only the entosepta have any ordinal significance. Until the adult condition is reached the exosepta are the temporary predecessors of the entosepta. The developmental relationships between the entosepta and exosepta are closely comparable with

those between the entotentacles and exotentacles. The law of substitution, first discovered by Lacaze-Duthiers for the tentacles of Hexactiniæ, is thus found to hold also for the septa.

8. Where the cyclic hexamerism of a corallite is incomplete the ordinary cyclic formula does not express the true relationships of the septa; the entosepta and exosepta vary in the same degree, so that the true morphological septal formula for a corallite with three cycles and part of another is $6, 6, X, 6 + 6 + X$, where X may be any number from 1 to 12.

9. The cycles of septa and mesenteries represent so many distinct recurring phases of growth at intervals all round the polyp, not a continuous increase from one extremity to the other as in metameric animals. With the exception of the first the members of each cycle follow a dorso-ventral succession, display a bilateral symmetry for some time, and ultimately assume an approximate radial plan. The succession for the third cycle of entosepta is two-fold.

REFERENCES.

Delage, Y., and Hérouard, E.

'01 Les Cœlentérés. Traité de Zoölogie Concrète, tom. II., Pt. 2. Paris.

Duerden, J. E.

'02 West Indian Madreporarian Polyps. Mem. Nat. Acad. Sciences, vol. VIII., 7th Mem.

Faurot, L.

'95 Études sur l'anatomie, l'histologie et le développement des Actinies. Arch. de Zoöl. Exp. et Gen., ser. 3, vol. III.

Gardiner, J. S.

'02 South African Corals of the Genus Flabellum, with an account of their Anatomy and Development. Marine Investigations in South Africa. Vol. II., Cape Town.

von Koch, G.

'81 Mittheilungen über das Kalkskelet der Madreporaria. 1. Das Vermehrungsgesetz der Septen. Morphl. Jahrb., bd. VIII.

von Koch, G.

'82 Ueber die Entwickelung des Kalkskeletes von Astroides calycularis und dessen morphologischer Bedeutung. Mitt. a. d. Zoöl. Stat. zu Neapel, bd. III.

von Koch, G.

'97 Entwickelung von Caryophyllia cyathus. Mitt. a. d. Zoöl. Stat. zu Neapel, bd. XII.

Lacaze-Duthiers, H. de.

'73 Développement des Coralliaires. Deux. Mém. Actiniaires à Polypier. Arch. de Zoöl. Exp. et Gén., tom. II.

Lacaze-Duthiers, H. de.

'94 Faune du Golfe du Lion. Evolution du Polypier du Flabellum anthophyllum. Arch. de Zoöl. Exp. et Gén., 3 ser., tom. II.

Lacaze-Duthiers, H. de.

'97 Faune du Golfe du Lion. Coralliaires. Zoanthaires Sclérodermés. Arch. de Zoöl. Exp. et Gén., 3 ser., tom. V.

Milne-Edwards, H., and Haime, J.

'57 Histoire Naturelle des Coralliaires ou Polypes proprement dits. Paris, 1857–60.

Schneider, A.

'71 On the Structure of the Actiniæ and Corals. (Translated by W. S. Dallas from the Sitzungsbericht der Oberhessischen Gesselschaft für Natur- und Heilkunde, March 8, 1871) Ann. Mag. Nat. Hist., 4 ser., vol. VII.

Semper, C.

'72 Ueber Generationswechsel bei Steinkorallen und über das M. Edwards'sche Wachsthumsgesetz der Polypen. Zeit. f. Wiss. zoöl., bd. XXII.

Verrill, A. E.

'01 Variations and Nomenclature of Bermudian, West Indian and Brazilian Reef Corals, with notes on various Indo-Pacific Corals. Trans. Conn. Acad. Science, vol. XI.

EVOLUTION IN A DETERMINATE LINE AS ILLUSTRATED BY THE EGG-CASES OF CHIMÆROID FISHES.

BASHFORD DEAN.

Recent attempts to explain evolutional processes, whether, *e. g.*, by natural selection, orthogenesis or use-inheritance, have been based, with but few exceptions, upon parent and offspring, in the direct relation of one to the other. Thus, (1) in the newly developed field of biometrics, variations in parent and offspring, have been discussed in terms of precision; (2) in embryology and embryopathology, ingenious experiments have tested the latent possibilities of the offspring at different stages in its career, and (3) on the side of paleontology, variations of progeny and parents have been examined on a giant scale in terms of survival and obliteration of masses of individuals. On the other hand observations are scanty, even in the present outburst of literature, which test the relation of the young to its parents by *indirect* means. This is, none the less, a line of inquiry which bids fair to become a fruitful one. And we may even at the present time consider what materials can be obtained which shall provide a series of parallel changes between the offspring and, *e. g.*, *some other product* of the parent, and through such means furnish, as it were, a *point d'appui* for evolutional studies. Thus: Are there any means of ascertaining whether an animal in providing for its progeny can produce structures *which form no organic part of the young yet which at the same time indicate accurately what the young will need in the distant future. And if these structures do occur, are they sometimes so special in their nature that they cannot be interpreted as general provision for the embryo, but rather for the late and complicated needs both of the genus for which they are provided and even of the species?* Obviously the parent has physical continuity with its young, but has it more than this? Has it the power to anticipate accurately the needs of the young, which it abandons and with whose subsequent fate it

clearly has nothing to do? Can it, for example, start an egg on its course of development and then provide it with a capsule whose special structures shall "foresee" accurately what the young is to become? Can it form a capsule which shall have the power, in spite of its lifeless substance, to develop as the enclosed egg develops, so that at each period it can best serve the corresponding stage of the embryo's growth? Such instances, if they can be found, are evidently of value in the examination of the complex problems of heredity.

The following notes are given since they appear to illustrate an extreme case in point: and since they also indicate that somewhat similar "purposeful" conditions may be demonstrated in the secondary embryonic membranes of other forms, *i. e.*, that these structures may be found the better adapted to the *future* grade of development of an embryo than has hitherto been suggested.

In examining the shark-like fish, *Chimæra* (*C. colliei*), the egg-capsule was found to be specialized, *i. e.*, adapted for the embryo at a late stage of development, rather than for the egg, in the following characters (Cf. figures in Biol. Bulletin, 1903, Vol. IV., pp. 271, 272):

I. *In Size.* — The capsule is not fitted to the egg; in fact, the outline of the egg is squeezed into a long ellipse, in order that it may be contained in the case, for if the case be opened and the tension relaxed the egg regains its circular outline, and the capsule with its appendage is then much (about nine times) longer than the egg. The great size of the capsule (longer in proportion to the adult body than that of a single egg of any known animal) is in evident adjustment to the advanced development of the young fish at the time it is cast upon its own resources. In point of fact it is then well grown — about one fifth the length of the adult — and by this time it has entirely lost its yolk and resembles the parent in remarkable detail — in axial and appendicular skeleton, in fins, viscera, in dermal, even in secondary sexual characters, *e. g.*, clasping organs of male.

II. *In Shape.* — The capsule as laid down in the oviduct is divided into special regions destined to contain the snout, trunk and tail of the young (at time of hatching). In earlier stages

the egg was contained in but a single region (trunk region) of the capsule. In a word, the capsule is destined to fit the shape of the embryo when full grown as accurately, to seek for a comparison, as the mummy case its mummy.

III. *In Permanence.* — The development of the young *Chimæra* is slow, taking many months, perhaps a year in hatching. The capsule is adapted to future conditions in its shape and texture, enabling it to resist wear and tear as well as the softening action of the water. Its "life," in other words, corresponds with the duration of the embryo's period of incubation.

IV. *In Attachment.* — During its long service the capsule is insured against displacement and attendant injury by means of specialized structures. These include a definite stalk, long, tough, springy, and a disc- or bulb-shaped terminal, capable of durable attachment.

V. *In Position.* — Experiments with capsules containing eggs show that when there is even a slight movement of water the capsules, as they lie on the bottom, assume a position with the keel side upward. This is the side which is dorsal in the orientation of the embryo and which bears the lid through which the fish later escapes. Furthermore, the capsule is provided with a vertical and a pair of lateral expansions which, if a current of water is present, cause it to rotate like a weather-vane in the direction of the oncoming stream. By this means the young fish is adjusted to its respiratory current and at the same time protected against the injuries which would follow the continual dislodgment of the capsule.

VI. *In its Orientation with Respect to the Growing Embryo.*—The germinal disc and early blastoderm in about fifty capsules examined occupied a constant position with reference to the capsule, appearing on the keeled side, and when the young embryo could be determined the head always pointed in the direction of the large end, *i. e.*, the operculate end, of the case. The same position is maintained in later stages, the head directed toward the large end of the capsule, the tail gradually invading the narrow sheath-like end. Symmetry is maintained with reference to the sagittal plane of the capsule.

VII. *In its Arrangement Enabling the Imprisoned Embryo to*

Obtain a Circulation of Water. — This the capsule provides by means of an elaborate system of perforations. These are bilaterally arranged, one series situated directly above the future gill region of the embryo, and a second series in the sheath in which the tail develops. These rows of perforations, moreover, are graduated in size, suggesting that in certain regions the perforations are adapted to functional needs. The embryo causes a water current to enter above the gills and to pass out on either side of the tail. (Cf. the turning of the capsule against the current, like a weather-vane.) In this connection the writer notes that he has seen the embryo execute vigorously a rhythmic undulating movement of its greatly elongated tail, which, provided with a continuous dorsal-caudal and anal-caudal fin, can only be interpreted as a specialized organ for forcing the water backward through the case.

VIII. *In its Elaborate Provision for the Escape of the Embryo at Term.* — By a delicate adjustment of the folded dorsal wall of the capsule, a line of separation is formed in the respiratory perforations above the gill-region. Thus arises an opercular flap, which finally opens in a way which suggests the opening of the lamellar beak of a duck, and thus enables the young fish to escape. This arrangement is a remarkably complicated one, the valve being provided, among other things, with roll-over margins which preclude its being opened from without and so perfectly adjusted that it opens only to the necessary degree. Once opened, moreover, the valve margins cannot be restored in their former relations, thus indicating that a state of tension has existed, comparable somewhat to that of dehiscent seed capsules.

IX. *In the Progressive Changes in the Lifeless Substance of the Capsule which take place pari passu with the Developing Needs for these Changes on the Part of the Growing Embryo.* — At the time the capsule is deposited it admits no water to its interior. In fact, the unused tail sheath is then entirely filled with a plug of thick albumen. The perforations are formed by a process of weathering, during which delicate septa obstructing the openings are gradually broken down and thus a greater current of water is admitted to the interior of the capsule. In later stages these openings are largest and most numerous. By the same process

the opercular flap, *i. e.*, the opening valve, is prepared progressively, so that the embryo can escape in due time.

X. *In its Provision for the Latest Embryonic Characters in Different Species.* — The egg capsules of four species of *Chimæra* known to the writer, present distinctive characters, and among these are some which are prophetic of the structures of the adult fish. Thus the tail sheath of the capsule of *C. colliei* is thick and relatively short, fitting the tail of the embryo of a species in which no adult opisthure is noticeable: in *C. mitsukurii*, on the other hand, the opisthure of the adult is of extreme length, and the tail sheath of its egg capsule is correspondingly long and delicate. The egg cases of *C. phantasma* and *C. monstrosa* suggest correctly intermediate conditions in the adult opisthures.

To return to the theme of the present paper: A point of general interest presented by this complicated capsule is its bearing upon the factors of evolution. For, considering always that the substance of the capsule is only indirectly connected with the egg, *i. e.*, as a secretion formed by the parent after the mechanism of heredity has already been established in the egg, it nevertheless (1) "foresees" with startling exactness the size and shape of the young fish when many months hence it comes to hatch out, and (2) it provides a series of progressive modifications adapted to the developing physiological needs of the young. It is evident, accordingly, that if natural selection be adduced to explain the present phenomenon it encounters difficulties more numerous and complex than in usual instances. In the latter cases selection concerns itself with variations which affect the progeny directly; but in the present case variations must have occurred in the lines *both* of the progeny and, indirectly, of its far less individual capsule-forming capabilities — with the result that a succession of closely correlated steps in variation must have coincided in both distinct directions.

To make more apparent the complicated nature of this procedure an example might be devised in which numbers appear. Thus: At each point in the development of the young *Chimæra* let the number of important fortuitous variations be represented by 1,000 (which I think all will agree is a number of possibilities

modest rather than excessive). And let the same number of possible variations be present in each of the various stages in the development of the capsule. Accordingly, at the first stage let us grant that variation number 673 happened to be the most favorable one: this then was selected in both the embryo and in the capsular secretion; at the second step some number, *e. g.*, 973 was similarly selected in both; at the third, number 14 in both; at the fourth, number 467 in both; at the fifth, number 801 in both; and at the two hundredth, number 761 in both. In other words, if we reckon the progressive series as extending over but two hundred steps, taking this number merely for the sake of example, as the possibilities are multitude, the chances are obviously remote of establishing the foregoing coincidences in the two series (one representing the development of the egg, the other of the capsule) by constantly repeated selection of fortuitous and fluctuating variations. For, taking the first coincidence of a favorable fortuitous variation of the embryo selected by a favorable fortuitous variation in the capsule, or indeed, even a coincidence of variations which are passable, not optimum, in both, it is evident that we are dealing with an accident of an improbable character. Thus, for variation 673 of the embryo to have seized upon variation 673 of the capsule there is but one chance in a thousand. Granted, however, that this unexpected result happened: for the second optimum variations in egg and capsule to have coincided, say the number 973 in both, there is again but one chance in a thousand, — or in other words, the chance for coincidence in the favorable selection of the optimum fortuitous variations one and two, the chances would be one in a million; of variations one, two and three, one in a billion.

It would be urged, on the other hand, by selectionists, that these figures are misleading, since with hosts of variations taking place in each stage of egg and capsule there would always be a coincidence of one favorable (although perhaps not the best) variation in the egg with a correspondingly favorable (perhaps not the best) character in the capsule. But this assumption leads to no essential change in the result, for in this case if the variations do not correspond with fair accuracy, at successive stages the embryo will not fit the conditions of the capsule, and

must accordingly perish.[1] In other words, in the first stage of variation, assuming again variations 1 to 1,000 in egg and in capsule, the chances for coincidence of even passable variations would be so small that a vast proportion of the embryos would fail to mature. And of the small number which did survive the first selection the majority would evidently be eliminated in the second stage of selection. For one egg, in short, to have run the gauntlet of the favorable variations in the capsule there must have been, and must still be an enormous proportion of failures.[2] The latter is evidently false, for it was found that the eggs of *Chimæra* are about as hardy and fruitful as the eggs of other sharklike fishes. Morever, if it is necessary to show still further the insufficiency of this selectionist argument, one need only reflect that nature would have to support millions of adult *Chimæra* in order that there might be laid one golden egg! The race, in short, could at the best have existed but a few generations in view of its slowness of breeding and the small number of eggs (possibly a dozen a year) laid by each female. But this inference is palpably false, for we know that the race of chimæroids has been producing specialized egg capsules from Jurassic times.

Natural selection of fortuitous variations is, accordingly, clearly valueless in explaining the evolution of the present capsule. Use and disuse also are to be eliminated as factors in its development. If a neo-Lamarckian suggests that the capsule is a result of use, having been primitively moulded *in utero* around the *full grown* young, one need only reply that such a suggestion is in itself anti-Lamarckian, for why would a capsule, let alone a complicated one, be formed at all when the embryo is so fully grown that it cannot need it, and why should elaborate provision for aquatic breathing be present in a uterine capsule? One need hardly offer

[1] Thus, the proper variation for the opercular lid can only follow the favorable variation for the respiratory pores in the capsule, and these again can only follow if the capsule is of a great size, definite shape, or durable structure. And, of course, as definite an order must be followed in the stages of the embryo. And finally, the accurate regulation of time between the two must coincide. Thus the breathing pores of the shell must not open too early or too late.

[2] Admitting that "favorable," rather than optimum variations could have been successfully selected. Even organic selection, it seems to me, reduces the difficulty only in unessential regards.

the objection that in uterine development the well known law is to reduce secondary membranes rather than to increase them.

The capsule of *Chimæra* must stand, I conclude, as an instance of evolution in a determinate direction. Unhappily, however, it gives no data by which the cypher of orthogenesis can be simplified.

NEW FACTS CONCERNING BOTHRIOLEPIS.

WILLIAM PATTEN.

In the following paper, I shall present a preliminary account of my observations on a large collection of *Bothriolepis* recently obtained in New Brunswick. A more complete and fully illustrated account will be presented later.

This new material shows structural features heretofore unknown, and much that one could hardly hope to see preserved in any fossils, especially in ones so old as these.

The trunk, Fig. 1, was very slender and covered with a soft skin devoid of scales or of any other markings except those mentioned below. In spite of its delicate structure it is often only moderately compressed, or distorted. In the region of the posterior dorsal, it may present a somewhat triangular cross section, resembling that of *Cephalaspis* in a corresponding region, but without any traces of a lateral fold or of fringing processes.

A few small irregular plates, with the typical sculpture of the buckler, are imbedded in the skin along the dorsal surface, immediately in front of the anterior dorsal, and numerous minute ones are scattered irregularly over the flanks in the same region.

One specimen shows indications of a lateral groove, and, dorsal to it, a few oblong folds suggestive of segmentation.

The anterior dorsal fin is low and elongated, the posterior one very high and rounded. Both fins are often preserved with wonderful clearness, but show no other detail than a faint striation probably due to the presence of delicate subdermal rays.

The elongated tail, with its axis slightly curved, terminates in a narrow band. The dorsal margin consists of a delicate membrane, strengthened by a row of curved rods lying close together and arranged with great regularity. The basal ends of the rods are swollen, and one is turned a little to the left, and the adjacent one, to the right, of the median line. The rods extend on to the ventral margin of the terminal band, into the ventral lobe. The latter is faintly striated like the dorsal fins. Its anterior ventral margin appears to divide, as though it were continued forward

into the lateral folds, although no such folds have been detected in the trunk region.

There are no indications whatever, in either surface views or in sections of vertebral centra or arches, and the preservation of the specimens is so perfect, that there is every reason to believe such structures, even if formed of cartilage only, were absent. Neither have we found any indications of a notochord, although one may infer from the outline of the trunk that a notochord was present. It was probably surrounded by a membranous sheath of no more consistency, if as much, than that in *Amphioxus*.

Several specimens show a remarkable membranous frill protruding from the ventral and lateral margins of the posterior opening of the buckler. It extends completely across the ventral surface like a curtain, and is entirely separate from the overlying trunk. The lateral margins vary a good deal in different specimens, but, when clearly shown, they extend backwards in long lobes, which in one specimen are clearly thrown into regular folds like those indicated in the restoration. On the sides and toward the dorsal surface, the membrane appears to be reduced to a narrow fringe projecting from the inside margins of the opening of the shield, Fig. 2.

The ventral surface of the trunk extends without interruption or attachment over the frill and for about an inch over the visceral surface of the posterior ventrals. Here there is a prominent transverse ridge and a large scar on either side, probably the places of attachment of the trunk to the shield.

A large but very thin and nearly circular scale, or plate, marked with deep-lying radiating lines and with superficial concentric ridges is attached to the ventral surface of the trunk, between it and the inner surface of the posterior ventrals. The opening to the cloaca lies just above this plate, Fig. 2.

All the sectioned specimens showed the presence inside the buckler of a peculiar core, about an inch and a half long and half an inch in diameter, Fig. 2. It varies in shape in different individuals, but in all of them it has essentially the same characters and the same location in an antero-posterior direction. But its position shifts from side to side as though it were suspended in a rather large space and free to move laterally, or in a dorso-

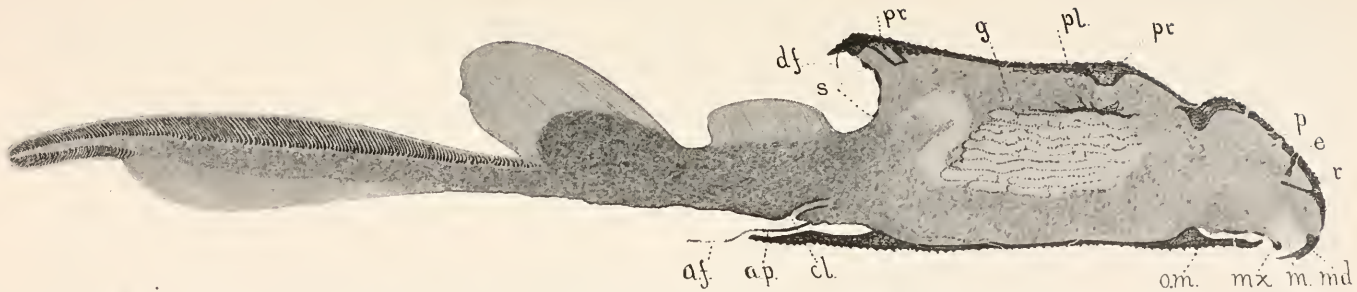


FIG. 2. Median section of *Bothriolepis*. *A*, anal frill ; *a.f*, anal plate ; *cl*, cloaca ; *d f*, dorsal extension of anal frill ; *e*, ethmoid ; *g*, gill lamellæ (?) ; *m*, mouth ; *md*, mandibles ; *mx*, maxillæ ; *o.m*, oral membrane ; *p*, pineal pit ; *pl*, bone-like plate ; *pr*, processes for attachment of internal structures ; *r*, rostral plate ; *s*, ridge scales.

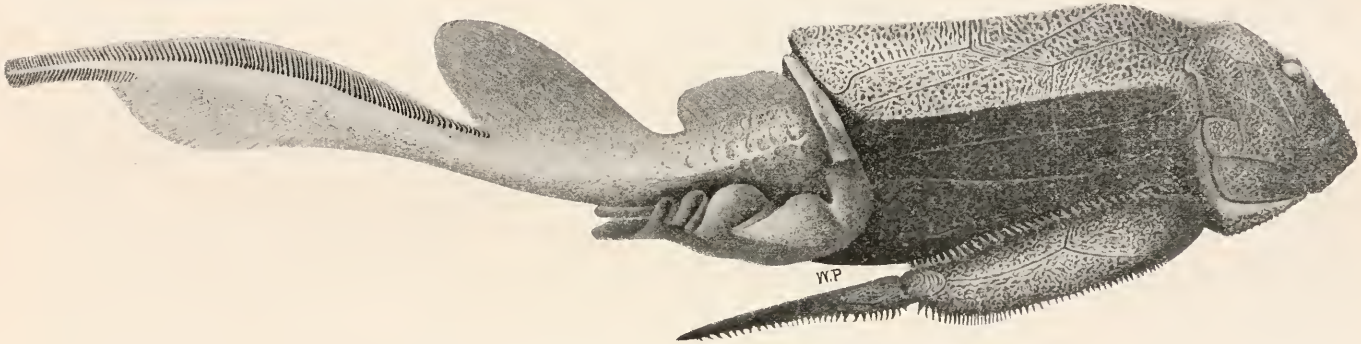


FIG. 1. Reconstruction of *Bothriolepis* seen from the side.

ventral direction. Its anterior end lies back of the cervical suture and from there it extends backwards about two thirds the length of the post-cephalic portion of the buckler. It is readily recognized by its fine, soft matrix and the concentric black lines, each line clearly and sharply defined and separated from the adjacent ones by regular intervals. The lines are similar in color and thickness to those made by the skin when seen in section. The whole structure, while puzzling, is unquestionably produced by some organic structure, not by sedimentation within an irregular cavity, or by mud accumulated within the alimentary canal. The anterior boundaries of the black lines are ill defined, but the posterior ones often form distinct loops as though the whole structure consisted of a series of broad lamellæ wrapped around a central axis, and with free, more or less separated, posterior margins. In some cases the whole mass of lamellæ is much distorted, or they may protrude from some rupture in the walls of the crushed shield. Under these conditions they still preserve their essential characters, showing that while originally soft and flexible they had at least as much firmness as the skin of the trunk. Behind the laminated portion, the soft matrix extends in an irregular undefined and structureless mass toward the cloaca. The remaining space within the buckler may be filled with a coarser matrix similar to that in which the whole animal is imbedded.

On the dorsal side of the laminated body, there are usually scattered fibrous masses, and one or more irregular, undefined bony plates, apparently attached by vertical sheets of blackened tissue to a low median ridge on the inner surface of the anterior median dorsal. Anteriorly, this ridge deepens into a prominent hollow process directed downwards and forwards toward the thickest part of the covering on the laminated core, Fig. 2.

Several specimens have been found with the mouth parts in their natural position, held there by membranes, whose contours can be determined with considerable accuracy.

The oral region, Fig. 3, is covered by an undulating structureless membrane in which are imbedded the various oral plates. The membrane is attached to the lateral and anterior margins of the head, as far at least as the shoulder on the anterior border of the mandibles. It extends backwards, underneath the anterior

ventro-laterals, as far as the large transverse ridge on their inner surface, to which it seems to be attached. It extends across the median line without interruption except between the mandibles. It also appears to be absent between the mandibles and maxillæ, Figs. 2 and 3, *o.m.*

The mandibles are thin, concave plates of bone, continuous with the oral membrane on the sides and in front, but with free

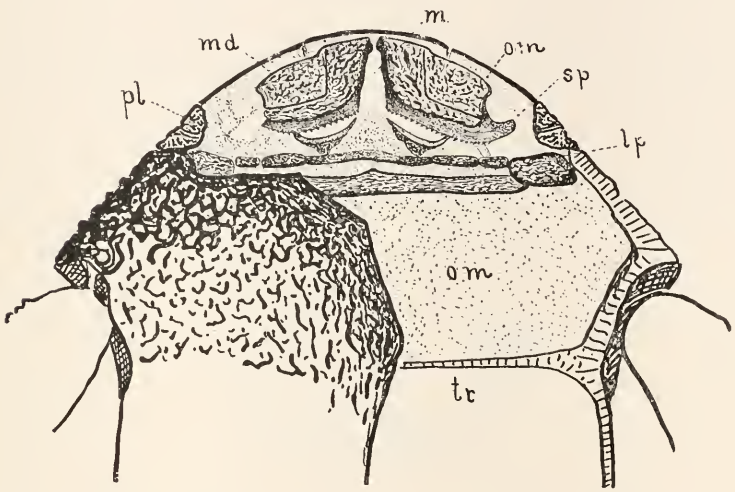


FIG. 3. Ventral side of head, with the anterior ventro-laterals removed on one side, to show extension backwards of oral membrane to the transverse ridge, *tr*; *l.p*, lateral plate attached to the two transverse bars; *m*, mouth; *md*, mandibles; *o.m*, oral membrane; *pl*, prelateral; *sp*, spur for attachment of muscles. × 1½.

median and posterior margins. The exposed surface presents the characteristic sculpture and the well known sensory groove. The posterior margin is nearly smooth and sharply bevelled, ending in an extremely thin edge nearly the whole length of which is broken into irregular serrations. The lateral and central serrations have more or less truncated points, the median ones are more regular in shape, sharply pointed and directed diagonally backward and inward toward the median line. The median margins of the mandibles are very thick and are provided with two horizontal edges, Fig. 4, *B*. Anteriorly the innermost edge becomes nearly vertical, forming a sharp-edged, tooth-like process, rounded in outline when seen from the side, Fig. 3. The plate

said by Woodward to unite the two mandibles does not exist. The plate seen by him was without doubt the displaced thin shelf of bone projecting backwards from the inner surface of the premedian or ant-orbital, Fig. 2.

The notched anterior margins of the mandibles are uniformly thin and rounded, and are continuous with the oral membrane, which unites them with the margin of the head, except possibly for a short space near the median line. The lateral margin is narrow and rounded where it becomes continuous with the oral membrane. A broad spur extends laterally on the visceral side of the membrane (Fig. 3) serving no doubt for the attachment of muscles.

The visceral aspect of the mandibles is nearly smooth, except for what appear to be some indistinct muscle scars and a very

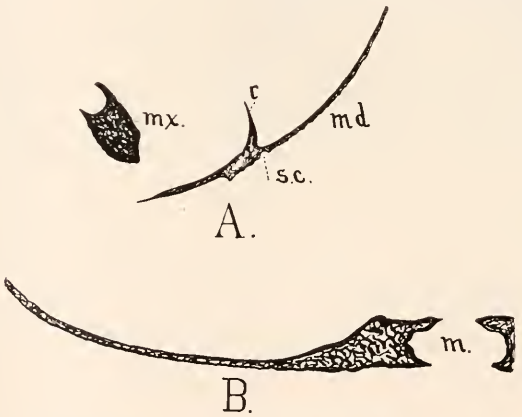


FIG. 4. *A*, sagittal section through mandibles and maxillæ, a little to one side of the median line of the head; *md*, mandibles; *mx*, maxillæ; *r*, internal muscular (?) ridge; *s.c*, sensory canal. *B*, transverse section of the mandibles to show heavy median margins. × 5⅓.

prominent sharp-edged ridge, which extends the whole length of the mandibles, Fig. 4, *A*, *n*.

The maxillæ are peculiar S-shaped plates lying behind, or more requently underneath, or dorsal to, the mandibles, Figs. 3 and 5. The median arm of each maxilla usually stands so as to expose a nearly smooth rounded ventral edge and a finely ornamented, somewhat triangular, strongly convex posterior surface. The median margin is undulating in outline and with a smooth border. In

sagittal sections it is seen to be deeply concave on its median posterior face, Fig. 4, *A*, *mx.*

The lateral arm of the maxilla is a narrow band, dull black and smooth. In the position shown in Fig. 3, it lies below the oral membrane with its concave surface directed upward and forward.

At the union of the two arms the band is twisted through an angle of about 45°, so that when seen directly from behind the ventral edge of the median arm forms a nearly straight line, while the lateral arm is concave on its ventral surface and on its posterior margin, Fig. 5, *A* and *B*.

The position and structure of the mandibles clearly indicates

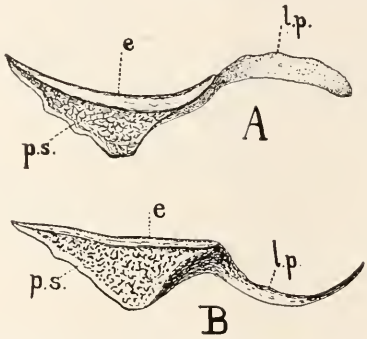


FIG. 5. *A*, maxillæ seen from ventral side and a little from behind. *B*, same seen directly from posterior surface. × 4.

that each mandible moved independently to and from the median line, so as to bring their stout crushing and cutting edges into apposition. They are frequently found with their posterior margins widely separated from the body, or even thrown forward in front of the head with their ventral surfaces facing upwards. Thus it is extremely probable that the mandibles, like two great lids or covers, could swing forwards and backwards on the membrane attached to their anterior margins, although it is improbable that they normally passed beyond the vertical position during life, Fig. 2.

The maxillæ are usually widely separate in the median line, and each was probably quite independent of the other. Their form indicates that their movements were complex. They appear to have had a rotary movement on their long (transverse)

axis, thus swinging their ventral edges forwards and backwards in a nearly horizontal plane. At the same time their median ends could be thrown forwards and medianly, thus bringing their diverging median margins more nearly parallel, and nearer together in the median line. The difference in curvature, density and rigidity between the mandibles and maxillæ, and the direction of the marginal spines on the mandibles and their absence on the maxillæ makes it very improbable that one pair acted directly against the other. It is more likely that the maxillæ merely pushed the food forward and inward, where it could be crushed, or cut, between the stout margins of the mandibles, in a manner similar to that which obtains in the mouth parts of arthropods.

I regard the paired upper and lower jaws of *Bothriolepis* as the homologues of separate right and left halves of the embryonic mandibular and maxillary arches of the higher vertebrates. As is well known these ridge-like lobes appear in all vertebrate embryos on the sides of the head, close to the nerve cord and then migrate toward the median ventral side. These embryonic mandibular and maxillary lobes I have for many years regarded as the vestiges of arthropod appendages which, owing to the special conditions under which the vertebrate head is developing, are carried toward the hæmal side of the head, instead of the neural side.

Bothriolepis is the only fish-like animal in which the halves of the jaws are separate, and functionally independent in the adult, and it thus supplies precisely the condition which the arthropod theory of the origin of vertebrates demands.

Back of the maxillæ, the oral membrane is strengthened by two bands of dermal armor. They are very thin and delicately ornamented, and when the head is in a normal position the posterior band is entirely, and the anterior one partly, overlapped by the anterior ventral plates. The narrow anterior band consists of five or six segments. The posterior one is unsegmented. Both bands are attached to a large lateral plate, the lateral end of which is bent at right angles, and attached to the lateral walls of the head, Fig. 3.

A small quadrilateral plate, that I shall call the *pre-lateral*, lies

just in front of the extra-lateral. It should be regarded as belonging to the anterior dorsal margin of the head, Fig. 1. But it is movable and is nearly always folded over on to the ventral surface, Fig. 3. It is marked with a sensory groove, probably a continuation of the anterior vertical one, although it does not appear to be exactly in line with it.

The median plate of the ocular opening is nearly perforated by a deep pineal foramen, often indicated by a low tubercle on

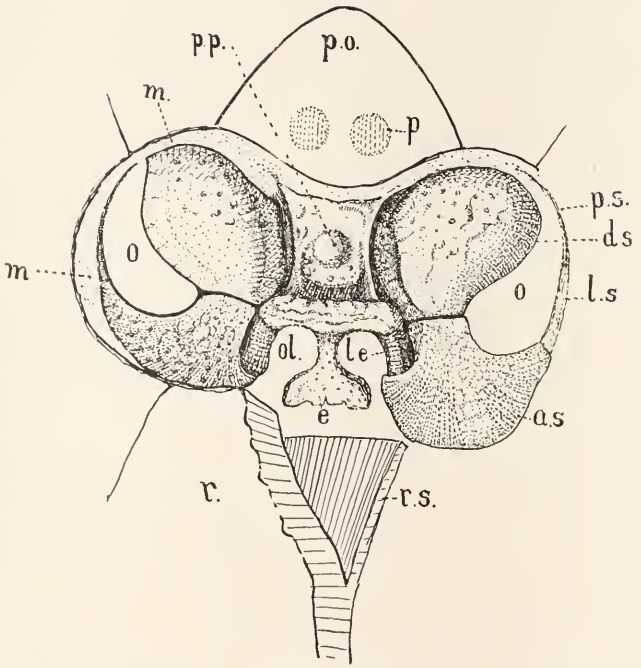


FIG. 6. Ocular and olfactory plates, in position with part of the rostral and lateral plates removed. *a.s*, anterior sclerotic; *d.s*, dorsal sclerotic; *e*, ethmoid; *l.e*, lateral arm of ethmoid; *l.s*, lateral sclerotics; *m*, membrane; *o*, corneal surface of eye; *ol*, olfactory opening; *p*, two pineal eye pits on under side of post-orbitals; *p.p*, pineal tubercle on outside of pineal plate; *p.s*, posterior sclerotics. × about 5½.

the outer surface, Fig. 6. A pair of similar pits, but not quite as deep and without any surface indications of their presence, are clearly shown on the inner surface of the small post-orbital. There is every reason to regard these three pits as similar in character and as indicating the presence of a tri-ocular median eye.

A broad thin shelf of bone extends inwards from the ant-orbital plate forming a wide chamber between the anterior surface of the T-shaped bone described by Whiteaves, or the ethmoid as I shall call it, and the two arms of the ant-orbital, Fig. 2. When the anterior face of the ethmoid is exposed, it is seen that its arms nearly enclose two circular openings, *ol*, in which the floor of the olfactory organ was probably situated, and which also served for the passage of the olfactory nerves.

These two pits clearly correspond to the antorbital fossæ of *Cephalaspis* and *Tremataspis*, and thus a more satisfactory explanation of the location of the olfactory organs is furnished than the suggestion that they are situated on the margins of the mandibular plates, where there are no indications whatever of any infoldings of the oral membrane that might be regarded as olfactory organs.

Two large saucer-shaped sclerotic plates are attached by short movable arms to the lateral arms of the ethmoid, *a. s.*, and two much thinner and crescent-shaped plates, *l. s.* and *p. s.*, form the lateral and posterior walls of the orbits. The well known plate attached to the sides of the pineal plate, *ds*, completes the dorsal wall of the orbit.

All these plates are held together, and to the sides of the sensory opening, by tough but flexible membranes, leaving thus a relatively large space for the movements of the various parts. It is clear from the position of the plates in different specimens that the pineal plate could be pushed backward for several millimeters drawing the ethmoid backwards and upwards so as to open the olfactory chamber. This movement would also raise the anterior and lateral sclerotics which in turn would elevate the dorsal sclerotics and expose the eye openings, *o*, on the sides and front. The reverse movement would lower the ethmoid and draw the corneal surface of the eye into a pocket formed by the thick median margins of the adjacent cranial plates. Thus the eyes and olfactory pits could be opened and closed by the same set of movements.

The sensory canals that converge toward the median occipitals do not unite there as usually figured. Their posterior ends separate close to the median line and terminate in two small open-

ings, evidently comparable with the openings to the so called endolymphatic ducts of *Trematáspis*.

In order to understand the morphology of the cephalic region of the Ostracoderms it is essential to know the location of the gills. Certain problematical structures found in the chamber in front of the ridge on the inner surface of the anterior ventro-laterals I formerly regarded as gills. I have not found any additional evidence in support of this conclusion. The presence of gills at this place would indicate that the extra-lateral was an operculum guarding the exit to the gill chamber, for it is the only cranial plate that could possibly act as such.[1] But its movements must have been very restricted and the place left for the passage of water extremely narrow, for both dorsal and ventral margins are articulated to the margins of the laterals and to the anterior ventrals respectively. The movement, such as it is, appears to be merely a tilting or partial rotation of the plate on its long axis in order to accommodate the slight dorso-ventral movement of the front part of the head, which would be impossible without some such arrangement.

On the other hand several facts indicate that the gills may have been situated in the post-cephalic portion of the buckler. These facts are (1): The presence of the superimposed lamellæ covered with what appears to be a continuation of the integument. These folds are more suggestive of gills than of any other known organs, and are almost invariably distinctly preserved, even where there is no trace of a notochord, showing that they must have had considerable power to resist decay, more, for example, than the folds in the mucous membranes of the viscera, the only other structures with which they might be compared. Moreover, if the walls of the viscera are as well preserved as this, then other internal organs should be visible in sections also, but as they are not, we are obliged to conclude that the folds in question are not produced by intestinal folds, or by any other structures of that

[1] The rather large opening in the sides of the anterior ventrals beneath the base of the pectoral spines, Fig. 1, could have hardly served any other purpose than for the passage of blood vessels and nerves to the pectoral spines.

The structure of the proximal end of the pectoral appendage indicates that some of the muscles moving the appendage were attached to the outer surface of the base of the appendage and to the outer surface of the anterior ventro-laterals.

nature. (2) The small size of the trunk at the posterior opening of the dorsal shield, and the presence of what appear to be dermal plates within the thoracic buckler, suggest that the trunk extends forwards within the buckler nearly to the head region, and that it is attached to it only along the low ridge and to the two large processes in the mid-dorsal line, to the posterior corner of the posterior ventro-laterals, where there are two large scars and a transverse ridge, and to the internal ridges near the junction of the cephalic and post-cephalic portions of the buckler. (3) The fact that the greater part of the buckler is usually filled with the same kind of matrix as that on the outside, while the part containing the finer matrix, and indicating the presence of organic material, forms a comparatively narrow band. (4) If the gills are located in this large chamber, the water must have passed out of the buckler at the posterior end, on either side of the trunk. Such a condition would harmonize with the fact that the cloaca is situated so far forward, for without such a current of water the excreta could not be easily discharged.

In conclusion, we may add that if the gills are located in the thoracic portion of the buckler, it becomes extremely probable that *Bothriolepis* possessed an atrial chamber similar to that of *Amphioxus*. The formation of such a chamber could have been brought about by the extension of the margins of the cephalic shield of such a form as *Cephalaspis* and their union on the ventral side, thus enclosing almost the whole ventral surface of the trunk from the anus nearly to the mouth, including the marginal appendages. The latter would then occupy about the same relative position as the lamellæ of *Bothriolepis*.

There has evidently been a forward extension of the ventral shield of *Bothriolepis* so as to partly cover the plates imbedded in the membrane of the oral region. The whole of this region, back to the ridge on the inner surface of the anterior ventro-laterals, with its four rows of plates, corresponds to the uncovered oral region of *Trematapis*, with its four rows of small plates surrounding the mouth.

HANOVER, N. H.,
June 21.

Vol. VII. *August, 1904.* *No. 3*

BIOLOGICAL BULLETIN

FORM-REGULATION IN CERIANTHUS, V.

The Rôle of Water-Pressure in Regeneration: Further Experiments.

C. M. CHILD.

Reduction of Water-Pressure by Means of Artificial Openings and its Effect on Regeneration.

Early in the course of my experiments the apparent relation between internal pressure and the rapidity of tentacle regeneration was noted. The facts obtained incidentally led me to believe that it would be possible to influence the course of regeneration by altering the internal pressure. The results of experiments have amply justified this belief, though I hope at some future time to be able to apply to the problem new methods which have suggested themselves, and thus to eliminate some of the complicating factors.

The problem was attacked experimentally by various methods which may be grouped under two heads: one group of methods consisted in the frequent reopening or spreading of the margins of an artifical opening in the body-wall, the other in the attempt to make an artificial opening in the body-wall of such a form that complete closure and consequent distension of the piece with water would be impossible or greatly delayed. The chief difference in the two sets of methods is that with the first, the pieces are reopened at stated periods, every alternate day, every day, or oftener, while with the second they remain undisturbed after section. The results obtained by these two methods differ little and afford strong evidence in favor of the view that water-pressure is an important factor in regeneration in *Cerianthus*.

Pieces Reopened at Intervals.

In experiments of this kind the results are more or less modified by certain complicating factors which it is difficult to eliminate completely. Some discussion of these is necessary before proceeding to an account of the experiments.

The region of the piece selected for opening was in most cases the aboral end simply in order that the region subjected to the irritation connected with repeated manipulation might be as far removed as possible from the regenerating region. As a matter of fact it was found that the manipulation itself, even though frequently repeated and including extensive section or tearing of the tissues, exerted little or no influence on the course of regeneration in other regions of the piece.

The description in the third paper of this series ('04*a*) of the process of inrolling of the body-wall after section in consequence of the elasticity of certain of its layers explains the necessity for repeated opening of a piece when continued communication between the enteron and the exterior is desired. In practice it was found that within a half hour after section the opening was usually reduced to mere slits and crevices by the approximation of its margins. Moreover, the ectodermal slime, which plays an important part in the provisional closure of wounds, as was described in the third paper ('04*a*), aids in plugging the small spaces between the approximated margins. In consequence of these two factors, the inrolling of the body-wall about a cut and the presence of slime which plugs the crevices, the attempt to keep the enteron in constant communication with the exterior through an artificial opening meets with great difficulties. The attempt was made to avoid these complicating conditions by introducing a glass tube into the body through the opening, but even when the tube was provided with a lip to hold it in place the pieces succeeded in creeping away from it, and when it was secured by a ligature the body separated at the ligature if this was drawn tightly, or gradually drew itself out if the ligature was loosely tied. All attempts to keep the end open by the introduction of some foreign body failed.

The only other possibility in pieces of such a form that closure is possible is the repeated opening and spreading apart of the

inrolled margins to permit escape of the water from the enteron. From what has been said above regarding the rapidity of infolding of the margins and the closure by means of slime, it is evident that this reopening must be frequent if the establishment of water-pressure in the enteron is to be entirely prevented. In the course of my experiments of this kind it was found practically impossible to prevent closure by approximation of the margins and plugging of the opening with slime between the times of opening. Pieces in which the mouth had regenerated, thus permitting rapid entrance of water, were often found more or less distended with water after being left over night. Frequently even a shorter time than this was sufficient to permit provisional closure and the establishment of more or less pressure in the enteron. It is clear therefore that unless such pieces are reopened at very short intervals — one to two hours — the establishment of some degree of water-pressure in the enteron cannot be prevented in many cases. It was impossible for me to reopen the pieces every hour or two during several days or weeks, consequently in no case was it possible to eliminate entirely water-pressure in the enteron.

As a matter of fact, however, the water-pressure in a piece in which one end is closed only by approximation of the margins and plugging of the crevices with slime is in most cases much below the pressure in normal animals, as could be readily determined by the resistance of the body-wall, the degree of distension, etc. It follows from this fact that pieces whose aboral ends are repeatedly opened and not allowed to close definitively by the formation of new tissue will be subjected to much less pressure than those allowed to close in the usual manner. It is possible then to reduce the enteric pressure if not to eliminate it entirely, and as my experiments will show, the reduction in pressure has a marked effect. In my experiments, the pieces were opened at intervals of from two days to half a day.

Except where statement to the contrary is made all experiments described here were performed on *Cerianthus solitarius*. Each series retains the number given it in my notes and the date of each observation is given. Comparison of the control pieces will show wide differences in the rapidity of regeneration in different series, due of course to the temperature of the water at the time of experiment (cf. Child, '03*b*).

Series 48.

November 21, '02. — Ten large species of *C. solitarius* were cut transversely a short distance aboral to the œsophagus (Fig. 1), the portions aboral to the cut being used for experiment. In five of these pieces the body was split longitudinally for half its length from the aboral end and oblique incisions were made at various points on the longitudinal cut surfaces (see dotted lines in Fig. 1). The object of this procedure was to produce great irregularity in the cut surfaces at the aboral end in order that the inrolled edges might not fit closely together but leave numerous spaces between them, thus permitting communication between the enteron and the exterior. These five pieces may be designated as the experimental pieces or the experiment.

The remaining five pieces were not injured aborally; they were placed in the aquarium under the same conditions as the first five and remained undisturbed, serving as a control.

From the beginning of the experiment to December 8 the experimental pieces were reopened on alternate days, *i. e.*, the slime was cleaned from the aboral ends and with the aid of needles and forceps the end was spread widely open, care being taken in each case to ascertain that the communication between the enteron and the exterior was unobstructed. From December 9 to December 17 they were reopened daily. The terms employed in describing the various stages are explained by reference to the first paper of the series (Child '03*a*).

November 28. — 7 days after section. Experimental pieces; no tentacles present; tentacular ridge not distinct; oral end not expanded.

Controls: one piece distended with tentacular ridge appearing; other pieces partly filled; tentacular ridge not distinct.

December 2. — 11 days after section. Experimental pieces: A few minute tentacle-buds on two pieces; two others with tentacular ridge just appearing. All four of these pieces have succeeded in closing aborally more or less perfectly and becoming more or less distended in the intervals between the reopening of the aboral end; the fifth piece remained widely open aborally and shows no trace of tentacles.

Controls: All with distinct tentacular ridge and some tentacle-buds just appearing.

December 8. — 17 days after section. Experimental pieces: Since these differ somewhat from this stage on, each piece is specially designated by a letter which it retains throughout the remainder of the experiment. In pieces *a* and *b* the longitudinal cuts extend only about half the length of the piece, in *c* about two thirds, while in *d* and *e* they extend more than three fourths the length.

(*a*) In this piece the longitudinal cuts are less deep than in the others and the end closes more perfectly being less irregular. At each examination before reopening the piece was more or less distended with water. Marginal tentacles 1 mm.; oral end partially expanded.

(*b*) This piece also succeeded in closing the aboral end more or less perfectly by approximation of parts between the times of reopening. Marginal tentacles 0.5–1 mm.; oral end partially expanded, a small area of new tissue visible at center.

(*c*) Has been only very slightly distended at any time: aboral end has remained partly open most of the time except for slime. The tentacular ridge bears only minute buds; the oral end is not expanded sufficiently to show the new tissue in the center.

(*d*) Has been widely open aborally since December 2: aboral end torn in reopening and margins rolled inward in such manner that a wide communication between enteron and exterior remains. The oral regeneration of this piece has not advanced visibly since December 2; the oral end is still unexpanded and new tissue not visible at the center; a tentacular ridge is barely visible but tentacle buds are wholly absent.

(*e*) Has remained most widely open aborally of all throughout the experiment: oral end wholly unexpanded; no tentacular ridge and no trace of tentacles visible.

Controls: All five pieces with oral ends expanded and showing a distinct area of new tissue at center; tentacles 1–1.5 mm.

December 12. — 21 days after section. Experimental pieces. Since December 8 have been reopened daily:

(*a* and *b*) Oral ends slightly expanded, diameter less than that of body; marginal tentacles 1.5–2 mm., slightly longer in *a* than in *b*; blunt and less slender than normal tentacles (Fig. 2).

(*c*) Oral end not expanded; very minute tentacle buds on tentacular ridge.

(*c*) Oral end not expanded; no tentacular ridge or tentacles visible.

Controls. Marginal tentacles 2–3 mm. (Fig. 3, one of controls).

December 17. — 26 days after section. Experimental pieces. Reopened daily since December 12, but *a* and *b* have been slightly distended at times. During the course of regeneration the mouth has formed in the usual manner, though more slowly

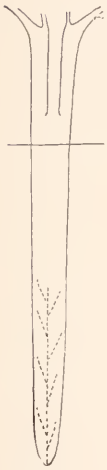


Fig. 1.

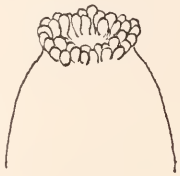


Fig. 2.

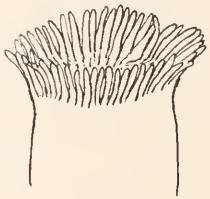


Fig. 3.

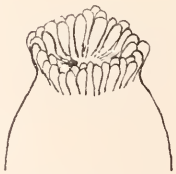


Fig. 4.

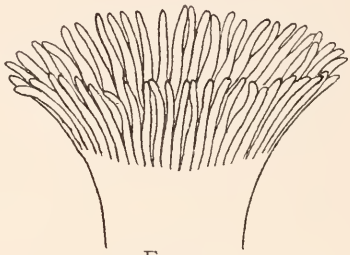


Fig. 5.

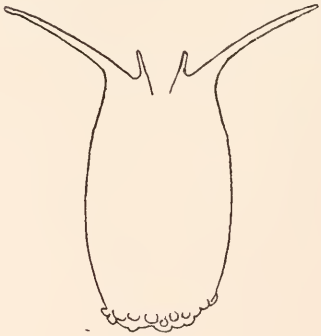


Fig. 6.

than in the controls, consequently water is now taken in much more rapidly than in the earlier stages.

(*a*) Oral end slightly expanded; marginal tentacles 3–4 mm., blunt and less slender than normal; no labial tentacles visible (Fig. 4).

(*b*) Oral end slightly expanded; marginal tentacles 2–3 mm., blunt and less slender than normal; no labial tentacles.

(*c*) Oral end not expanded; marginal tentacles 1–2 mm., blunt; no labial tentacles.

(*d*) Oral end not expanded; marginal tentacles 0.5 mm., no labial tentacles.

(*c*) No tentacles visible.

In all experimental pieces the diameter of the disc, where such is distinguishable, is, and has been throughout, less than the diameter of the body (Fig. 4), resembling in this respect the earliest stages of typical regeneration.

Controls. In four pieces marginal tentacles 6–8 mm. (Fig. 5), in fifth about 5 mm.; all with labial tentacles about 0.5 mm.

In all of the controls the diameter of the disk is greater than that of the body, the whole being somewhat trumpet-shaped like the normal animal.

From this time on the experimental pieces were left undisturbed in order to determine whether they still had the power to close and complete the retarded regeneration. The controls were discarded.

December 28. — (*a*) Aboral end closed by new tissue; body distended with water; marginal tentacles 5–6 mm., slightly more blunt than normal tentacles but much less blunt than at previous examination; labial tentacles 0.5 mm.

(*b*) Aboral end closed by new tissue; body distended; marginal tentacles 7–8 mm., still somewhat more blunt than normal; labial tentacles 1 mm.

(*c*) Aboral end closed by new tissue; body distended; marginal tentacles 6–7 mm., slightly more blunt than normal; labial tentacles 0.5 mm.

In all three pieces (Fig. 6) the diameter of the disc is still somewhat less than that of the body, but is relatively greater than on December 17, *i. e.*, the oral end is expanding. The aboral ends bear slight knobs and irregular masses of old tissue, which represent small shreds and strips of the old body-wall which had rolled into rounded masses during the experiment and are now undergoing absorption.

(*d*) New tissue at aboral end does not close end completely as yet; body is only slightly distended; marginal tentacles 1–1.5 mm., blunt; labial tentacles minute buds.

(*c*) Widely open aborally, about as before in appearance.

January 11, 1903. — (*a*) Body distended in normal manner

but still shorter and more sac-like than normal; marginal tentacles 12–15 mm.; labial tentacles 4–5 mm.

(*b*) Distended, form like that of *a*; marginal tentacles 10–12 mm.; labial tentacles about 3 mm.

(*c*) Distended, similar to *a* and *b* in form; marginal tentacles 7–8 mm.; labial tentacles 1–2 mm.

(*d*) Now completely closed and distended, similar to others in form: marginal tentacles 6–7 mm.; labial tentacles 1–1.5 mm.

(*e*) Has never closed aborally; oral end not expanded; minute marginal tentacle-buds visible; no labial tentacles.

Series 12.

September 12, 1902. — Six specimens were cut transversely just below the œsophageal region (Fig. 7). Three to be used as controls and three as experimental pieces.

September 15. — 3 days after section: In all six pieces the oral end was closed with delicate new tissue and all were partly filled with water. Three of the pieces were now split longitudinally from the aboral end for half their length (experimental pieces), the other three remaining undisturbed (controls). The experimental pieces contracted so strongly during this operation that the new tissue closing the oral end was ruptured, but the tentacle-forming region was not injured. The cut aboral ends of the experimental pieces are to be opened morning and evening of each day to prevent distension as far as possible.

September 19. — 7 days after first operation. Experimental pieces. Have not been distended at any time, although some accumulation of water has occurred in the intervals between the operations; the oral end of one not expanded and the new tissue merely fills crevices; two pieces with minute marginal tentacle-buds visible; in the third formation of tentacle-buds beginning at a few points on the tentacular ridge.

Controls: All well-filled with water and disc expanded; the new tissue at oral end forms a distinct central area in consequence of distension; one piece with marginal tentacles 2 mm.; two pieces with tentacles 1 mm.

September 22. — 10 days after first operation. Experimental pieces. Form of disc in all cases as before; two pieces with

marginal tentacles 1 mm.; third piece with tentacle-buds just visible.

Controls: All well-filled, with expanded discs; marginal tentacles in all cases 3–5 mm.; labial tentacles appearing.

The experimental pieces were now allowed to close and become distended.

September 24. — 12 days after operation. Experimental pieces. All distended, oral ends expanded but less so than in controls; all with marginal tentacles 2–3 mm.; one piece with labial tentacles just visible.

Controls: Marginal tentacles 5–7 mm.; labial tentacles 1 mm.

These two series are sufficient to serve as illustrations of the methods. In other series in which the same methods were employed the results were essentially the same, so that it is unnecessary to give the data for others in detail.

In a few series the new tissue at the oral end was punctured with a lancet as soon as it formed and then this end kept open by daily insertion of the lancet between the inrolled margins. Special care was taken in these operations not to injure the tentacle-forming region which lies a short distance from the cut margin (cf. the first paper of this series '03*a*), but to destroy only the delicate membrane and so allow communication between the enteron and exterior. It is unnecessary to give the details of this series since the results obtained are essentially similar to those of other series. In every case the diameter of the oral end remained less than that of the body, the appearance of the tentacles was delayed and the regeneration retarded as long as the pieces were kept open. Tentacle-buds when formed on open pieces were blunt and rounded, and after a time became whitish in color and opaque. When allowed to close the oral end began to spread in the typical manner and the tentacles grew as rapidly as in the controls.

Delayed Closure in Pieces of Certain Forms.

In my third paper (Child, '04*a*) some of the varieties of inrolling after section were described and it was shown that in pieces of certain forms, especially those which were split longitudinally, inrolling often occured in such manner as to delay or even pre-

vent union of the cut surfaces and thus the closure of the piece. Such pieces live for months in many cases, undergoing gradual closure from some region where approximation of cut surfaces renders possible the formation of new tissue between them. Sometimes the end or ends close readily but closure along the side is delayed and in cases of spiral inrolling closure of the end is possible only on the innermost part of the spiral and closure of the sides, not at all. These pieces present an almost infinite variety, but in all their forms afford important evidence as to some of the formative factors in *Cerianthus*. Without recognition of the method of growth of new tissue between cut surfaces, and the relation between tension due to internal water-pressure or other causes and growth of new tissue almost every one of these pieces might be described in detail — for scarcely any two are alike — as an "abnormality." If, however, we attempt to study the special conditions and the reactions to them in each case we find that the differences and "abnormalities" are not difficult to interpret. A number of pieces in which closure was prevented or delayed by the manner of inrolling are described below. These are selected from a much larger number which show various differences in detail from those described. I have endeavored, however, to select pieces which should show as far as possible the principal "abnormalities." The account will serve both as evidence upon the question of water-pressure and regeneration and as a description of the course of regeneration in the respective pieces. In most cases these experiments were performed without controls, so that it is impossible to determine exactly how much the first appearance of the tentacles is delayed; it will be sufficiently evident, however, that it is delayed. In the figures illustrating the inrolling of these pieces the lines employed for shading were so drawn that they also indicate in a general way the longitudinal striping of the body and thus show the direction in which the various parts are rolled inward.

Series 11.

September 12, 1902. — Three specimens were cut transversely a short distance aboral to the œsophagus (Fig. 7) and then split longitudinally on one side (Fig. 8). After section they were allowed to remain undisturbed.

September 19.—Seven days after section. Examination showed that none had closed, the cut margins rolling in on all sides having rendered closure impossible. Figs. 9 and 10 show two of the pieces as they appeared at this time. In the case of Fig. 9 the longitudinal cut separated both sides of the body at the aboral end and one of the lobes thus formed overlaps the other in the figure. The cut margins at the oral end are inrolled and united by new tissue much as in a cylindrical piece, but aboral to this region the two margins separated by the longitudinal cut have rolled in independently leaving a large open space between them. On the oral end are a few minute tentacle-buds — far below the normal number.

In Fig. 10 the inrolling has been somewhat different: on the right side of the figure it is much the same as in Fig. 9 but on the left, instead of rolling longitudinally the oral and aboral parts of the wall have rolled more or less transversely and in opposite directions. At the oral end only a part of the cut surface is exposed, the remainder being rolled inward behind the visible portion in the figure. The exposed portion of the oral end, corresponding to the longitudinally inrolled part of the body-wall is closed by thin new tissue where opposing cut surfaces are approximated in the inrolling, and bears a few minute tentacle-buds.

The third piece differs in detail from the other two as regards inrolling, but resembles them in that a few minute tentacle-buds are present and the enteron is widely open to the exterior.

In the first case (Fig. 9) the transverse section of each inrolled portion would show the form of a spiral with the longitudinal cut surface forming its innermost end: the same is true of the part on the right of Fig. 10, though here the inrolling is less complete. In these portions the contact between different parts of the body-wall is sufficiently close to permit a slight accumulation of water in the inner regions of the spiral and therefore a slight distension. The pressure in these parts never approaches that in the normal animal; as soon as it reaches a certain point escape of the water occurs between the approximated surfaces. Considering the tentacles, we find that their appearance is delayed. Cylindrical pieces closing in the typical manner regenerate tenta-

cles of the size shown in Figs. 9 and 10 in from four to five days at this season of the year. These pieces required seven days and the circle of tentacles is by no means complete. Only those portions of the oral end which are exposed and not completely collapsed bear tentacles.

These pieces were examined at intervals of a few days during three months.

November 1. —49 days after section. The pieces were much reduced in size owing to absence of food. During this time the tentacles had shown no farther growth, but had decreased in size so that they were scarcely visible. A part of one of the pieces

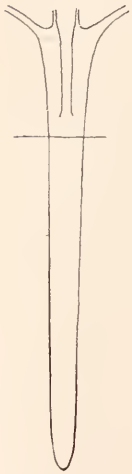


FIG. 7.



FIG. 8.



FIG. 9.



FIG. 10.



FIG. 11.

had constricted off, a not uncommon occurrence in cases of long continued collapse or severe injury.

November 10. — 58 days after section. It was found that the piece represented in Fig. 9 was finally closing. During the experiment the new tissue had been gradually extending from the ends of the longitudinal cut, slowly drawing the cut edges together, the last portion to close being near the middle.

The tentacles of this piece are now increasing in size again, being 0.5–1 mm. in length.

The other pieces have not closed, though in each some growth of new tissue along the cut surface has occurred. In these

pieces the inrolling occurred in such manner that the elasticity of the new tissue was insufficient to approximate the cut edges.

November 20. — 68 days after section. Tentacles of the closed piece 1–2 mm.; scarcely visible on other pieces, which are still open. Fig. 11 shows the closed piece as it appears at this time. The figure is drawn to same scale as Fig. 9, thus indicating marked decrease in size. The longest tentacles on the specimen are opposite the longitudinal cut; on each side of the cut the tentacles are very short; all tentacles are rather blunt. The new tissue uniting the edges of the cut is indicated by stippling.

This piece lived until December 28, and one of the others until December 12. During this time they become still farther reduced in size, but the second piece never closed.

Certain of the special features of this series require brief comment. It will be noted that even after closure the tentacles of the closed piece did not grow to any great extent, although some growth did occur. This failure to grow is probably due to the exhausted condition in consequence of absence of food. This may effect the power of growth, *i. e.*, the reactive capacity of the tissues, or it may cause a decrease in the water-pressure in one or both of two ways; first, it is quite probable that soluble products of katabolism accumulate in the enteron much less rapidly than earlier, owing to the decreased metabolism; if this is the case diffusion of water into the enteron and consequently distension of the enteron will be much less rapid: on the other hand there can be little doubt that the beat of the cilia is less powerful in these nearly exhausted specimens than in normal individuals. This being the case, it is easy to see that the inward current through the siphonoglyphe is not capable of maintaining as high internal pressure as in a normal animal: moreover, the local circulatory currents into the tentacles must also be less powerful. It is not improbable that all three of these factors play a part in the result; the osmotic factor is more important before the mouth is formed, decreased ciliary power afterward.

Series 17.

September 14, 1902. — Eight specimens were cut in the same manner as in Series 11 (Fig. 8) and allowed to remain undisturbed after section.

September 22. — 6 days after section. None of the pieces closed and none showed any tentacle-buds. On ordinary cylindrical pieces regenerating tentacles appear in about four to five days at this time of year and are usually 1 mm. long on the sixth day.

September 28. — 12 days after section. Four pieces possessed minute tentacle-buds on at least a part of the oral end. The pieces were variously rolled, those with tentacles at least in part longitudinally, the other four in part transversely. All were more or less widely open in the middle region. Two of the pieces at this stage are shown in Figs. 12 and 13. In Fig. 12 the portion on the right has rolled longitudinally in a spiral and at the oral ends the opposing margins of the spirally rolled portion are united by new tissue and bear minute tentacles. The remaining portion of the oral end is rolled aborally behind the tentacle-bearing portion, much as in Fig. 10.

In Fig. 13 the inrolling is almost wholly longitudinal and at the oral end new tissue has united the whole circumference. The longitudinal cut margins are rolled inward, but not united, though closely approximated. The circle of tentacle-buds is almost complete.

October 5. — 18 days after section. Six of the pieces are rolled transversely in a spiral, having changed somewhat in form since the last examination. In one of these in which the transverse inrolling began at the aboral end a few tentacles 2 mm. in length are present; in the other pieces there was no change as regards tentacles. The elongation of tentacles in this one piece was apparently due to the accumulation of some water in the outer turn of the spiral, *i. e.*, the more oral part of the piece, the margins of this turn being applied to the next inner turn closely enough to permit the establishment of some degree of water-pressure.

A few days later all eight of the pieces were dead, probably on account of accidental insufficiency in the water-supply.

Series 28.

September 26. — Nine specimens were cut transversely aboral to the œsophagus, then the aboral end was removed and the pieces

were slit longitudinally on one side. Thus these pieces resemble those of Series 11 and 17 (Fig. 8) except that a few millimeters at the aboral end have been removed. The object in the removal of the extreme aboral end in pieces was to avoid if possible the transverse inrolling from the aboral end which was so frequent in Series 11 and 17 (Figs. 10 and 12) and which interferes greatly with closure. In this series then the object was to

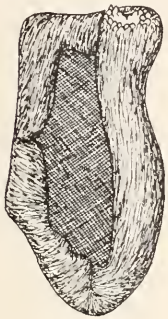


FIG. 12.



FIG. 13.



FIG. 14.

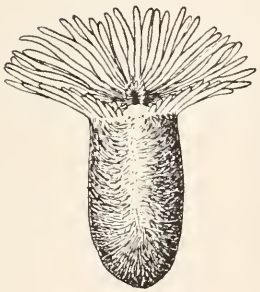


FIG. 15.



FIG. 16.

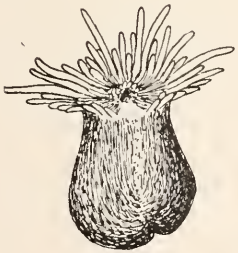


FIG. 17.

delay closure for a time but not to make it impossible. The experiment was continued for about four months and during that time all of the pieces closed and acquired essentially the typical form of the species.

October 3. — 7 days after section. None closed; variously rolled; only two with any traces of tentacle buds.

October 10. — 14 days after section. Six pieces with tentacles; three pieces still without any traces of tentacles.

From this point on it will be convenient to give the history of

each piece up to its closure separately. The pieces are numbered 1–9 in the order of their closure.

In each case the first date given for a piece is approximately the time when the elongation of the tentacles from the minute bud-like stage began. In every case then the regeneration of the tentacles has been retarded up to, or nearly to, this first date. In a few cases closure occurred a few days before the first date given so that the tentacles had attained a length of several millimeters by the time of examination. In almost every case the ends closed first and from each end the new tissue gradually extended along the cut towards the middle.

No. 1. October 10: Closed, oral end expanded, marginal tentacles 5–7 mm. on half of disc opposite the longitudinal cut, near the cut 2–3 mm.; labial tentacles just appearing on side opposite cut, not present on cut side.

October 17: Marginal tentacles 10–12 mm. on one side, 5–6 mm. on the other; labial tentacles 1–2 on uncut side, just appearing on cut side.

October 24: Marginal tentacles 12–15 mm., almost equal on all parts of disc except a short space without tentacles in region of cut; labial tentacles 3–4 mm., nearly equal on all parts.

No. 2. October 24: Closed; just becoming well distended; marginal tentacles 3 mm.

November 1: Marginal tentacles 6–8 mm.; labial tentacles just appearing, etc.

No. 3. October 10: Not closed by new tissue, but cut edges rolled in such manner that slight distension is present; marginal tentacles 3 mm., blunt and sac-like.

October 24: Still about as before.

November 1: Closed and distended. One marginal tentacle 6–7 mm., others 3–5 mm.; labial tentacles just appearing.

November 10: All marginal tentacles 7–8 mm., etc.

No. 4. November 1: Not fully closed by new tissue, but slightly distended and with sac-like tentacles 2–3 mm.

November 20: Closed and distended. Marginal tentacles 4–5 mm.; labial tentacles just appearing, etc.

No. 5. November 20: Just closed and becoming distended. Marginal tentacles 2–3 mm. on uncut side, reduced or absent on cut side (Fig. 14).

December 2 : Marginal tentacles 6–7 mm. on uncut side, 3–0 mm. on cut side ; labial tentacles present on uncut side, absent on cut side (Fig. 15).

December 12 : Marginal tentacles 10 mm. on uncut side, 5–0 mm. on cut side, etc.

No. 6. December 2 : Apparently not yet fully closed by new tissue, but slightly distended ; marginal tentacles 2–3 mm., blunt and sac-like.

December 12 : Apparently closed, distended ; one marginal tentacle 5 mm., others 2–3 mm.; labial tentacles just appearing.

December 24 : Marginal tentacles irregular in length, 3–6 mm., labial tentacles 1–2 mm., etc.

No. 7. December 6 : Closure by new tissue not complete, but slightly distended, some marginal tentacles 2 mm., sac-like, others mere buds (Fig. 16).

December 12 : Closed and distended. Marginal tentacles very irregular in length, 1–6 mm., absent near region of cut ; a few labial tentacles just appearing (Fig. 17).

December 24 : Marginal tentacles 3–7 mm., absent near cut ; labial tentacles 1–2 mm. on uncut side, a few absent on cut side.

No. 8. December 24 : Closed and distended. Marginal tentacles 4–5 mm., quite regular ; labial tentacles 1 mm., a few absent near region of cut.

January 21, 1903 : Little change since December 24.

No. 9. January 21 : Apparently closed, but distension slight, probably because of low temperature, slime, and continued starvation ; marginal tentacles 1–3 mm., mostly 3 ; labial tentacles 1 mm.

In this series the close relation between the internal water-pressure and growth of the tentacles is evident. Although the marginal tentacles appeared as minute buds before marked distension occurred, they did not advance beyond this condition in any case until the water-pressure increased — in some cases a period of more than three months. As soon as distension took place the tentacles continued their regeneration. This series constitutes a demonstration of the rôle of water-pressure as a factor in the regeneration of the tentacles, but does not throw any additional light upon the problem of their origin and localization.

Delay or prevention of closure was accomplished by various other methods and since the results in all cases agree with those of the series already described an account of all experiments along this line would be merely a multiplication of detail to little purpose. A summary of the principal other methods employed and the results obtained will be sufficient.

Series 31.

September 28. — Seven specimens were cut transversely aboral to the æsophagus as in Fig. 8 and then slit longitudinally down one side for three fourths of their length, differing from Fig. 8 in that the longitudinal cut did not extend all the way to the aboral end. These pieces closed much more readily than those of Series 11, 17 and 28. In this series as in Series 28 some of the pieces closed and became distended much sooner than others. The earliest distension of the body and elongation of tentacles occurred 14 days after section. Thirty-eight days after section all of the pieces were completely closed and with marginal tentacles more than 5 mm. in length. The delay in closure was not as great in most cases as in Series 28, but the results as regards delay in tentacle-regeneration agree in all respects with those of that series.

In two series —18 and 20 — 8 and 6 pieces respectively were separated by a transverse cut aboral to the œsophagus as in Fig. 7, and then the oral half of the piece was split into two parts by a longitudinal cut through both sides of the body. In consequence of this cut the oral part of the piece was doubled, the aboral remained single. In a few cases specimens with double oral ends and separate sets of tentacles resulted, but the more common results were the separation from the piece of the divided parts at its aboral end or the union of the two to form an individual essentially normal.

It will be evident at once, however, that the inrolling after such an operation must have been rather complex. Closure of the openings by new tissue was usually slow whatever the particular result, and delay in the regeneration of the tentacles occured here in the same manner as in the series alaeady described. The "double-headed" specimens will be described in another connection.

A similar splitting of the aboral end was accomplished in Series 19 and 38 — 6 and 4 pieces respectively. Three of these pieces produced specimens with double aboral end, but in the remainder the two parts united more or less completely. In these series delay in union of the cut surfaces also occurred, and whenever the presence of openings prevented distension the tentacle-regeneration was delayed, proceeding only when distension took place.

In several series the attempt was made to prevent union of cut surfaces at the aboral end by making the cut surface very irregular in form, splitting it into strips, making oblique cuts in it, removing portions, etc., the object being to bring about independent inrolling of different parts and thus prevent the complete closure of the end by new tissue. These experiments were not very effective, however. A greater or less delay in closure and a consequent delay in tentacle-regeneration was brought about, but in nearly every case closure took place within two or three weeks and after that regeneration proceeded in the usual manner.

General Considerations.

It is, I think, sufficiently demonstrated by the forgoing experiments that there exists a close relation between internal water-pressure and tentacle-regeneration. In order, however, to meet possible objections and to render more clear certain points a brief discussion of the results is necessary.

The objection may be made that the operations are severe enough to produce some pathological effect upon the pieces which prevents or retards their regeneration. Any objection of this nature is abundantly refuted by the data of the experiments themselves, from which it is clear that just as soon as the enteron is capable of holding water under some degree of pressure the course of regeneration becomes typical, and that even where the regeneration has been delayed for one or two months it is as complete when it does occur as if it had taken place immediately after section, although the resulting animal is smaller. Of course where the pieces are kept without food for periods of three or four months or more they become greatly reduced in size and their capacity for regeneration is diminished; nevertheless, even

such pieces as these show very clearly the result of internal pressure. A good example is the piece of Series 11 shown in Fig. 11 and the last pieces of Series 28.

In the preliminary survey in the preceding paper (Child, '04*b*) it was suggested that not only does the rapidity and amount of tentacle-regeneration depend to a greater or less extent upon the internal water-pressure, but the position of the regenerating marginal tentacles may be determined by the local pressure upon the inrolled oral end of the piece caused by the circulatory current which moves orally in the peripheral part of each mesenterial chamber and thus continually forces water into the blind sac at the oral end produced by the inrolling of the body wall and the mesenteries bounding each chamber. According to this view the tissue reacts to this local change in pressure by local growth and the position of the new marginal tentacle is determined. The first visible changes leading to regeneration consist in the reduction in thickness of the body-wall in consequence of the disappearance in large part or completely of the longitudinal muscle-layer. The local area of thin tissue being thus formed, it is effected both by the local pressure due to the circulatory current and by the general internal pressure. Both of these factors subject the area to tension and, as in other areas of thin "new" tissue (see the section on "The Closure and Distension of Pieces" in the preceding paper, Child, '04*b*), growth is the result.

A few tentative suggestions based upon the preceding data are added here in order to indicate the bearing of certain features of the experiments upon this hypothesis. But first of all the fact should be emphasized that water-pressure constitutes in any case only one factor in a complex process; it is impossible to doubt its influence in view of the experimental data, but recognition of the rôle played by water-pressure does not constitute a complete solution of the problem of form-regulation in *Cerianthus*.

It should be borne in mind that complete elimination of local water pressure due to currents has not been accomplished in any of the experiments. The methods employed have succeeded in bringing about a more or less complete reduction of the general distension of the body by water but the local circulatory currents

due to cilia continue of course wherever water is present in the intermesenterial chambers. These, however, must be considerably reduced in volume and force for several reasons: first, in the contracted condition of the open pieces the surface area of the intermesenterial chambers is much less than in distended specimens and the mesenteries are often so closely approximated that little water can enter the chambers; second, the entodermal surface is more or less folded and wrinkled in the contracted condition and so the number of cilia effective in producing currents is reduced; third, it is possible, though this is mere surmise, that the cilia beat less rapidly when the body is less distended, since the impeded circulation of water may reduce their supply of oxygen sufficiently to bring about such an effect. There can be little doubt then that the circulatory currents are at any rate reduced in volume and force in the open pieces. We find in such pieces a delay in the regeneration of the tentacles in comparison with pieces which are allowed to close and become distended in the usual manner. The fact that tentacles do finally appear in open pieces is due, according to this view, to the impossibility of eliminating completely by the methods employed the circulatory currents in the intermesenterial chambers. I hope in future experiments to be able to eliminate these currents more completely than has been possible with the methods employed thus far, and so to determine whether tentacle-regeneration can be completely inhibited by this means.

In open pieces the tentacles do not regenerate completely, but remain of small size as long as the pieces are open. As soon as closure and distension occur, however, they increase in length. The difference in the force of the circulatory currents in open and closed pieces may account for this difference. The assumption is justifiable that a certain degree of pressure causes a certain amount of growth, not indefinite growth, since the tissues as they grow apparently become less sensitive to the stimulus, or react to it in some unexplained manner so that growth gradually ceases. The so-called functional adaptation in bone, in the circulatory system, and in various other tissues indicates that this assumption is correct. Admitting this we may apply it to the case in hand and conclude that the failure of regenerating tentacles to grow

beyond a certain small size in open pieces is due to the slight degree of local pressure exerted by the circulatory currents.

In many open pieces the regenerating tentacles appear only on a part of the oral circumference or appear later on some parts than on others or grow more rapidly or to larger size on some parts than on others. Figs. 9–17 afford examples of all these conditions. In pieces which close and regenerate in the typical manner no such differences occur. All these irregularities in open pieces may be regarded as due to differences in the force and volume of the circulatory currents in different intermesenterial chambers. In parts which are much contracted or tightly rolled little or no water can enter, and consequently the circulatory currents are slight or absent. The fact that in cases of partial tentacle-regeneration the parts on which tentacles appear are more or less distended, while other parts are collapsed, confirms the conclusion. In general, cases of this kind are similar to those described in the preceding paper (Child, '04*b*) under the head of "Local Inhibition of Tentacle-Regeneration." In these cases, as in those described in that paper, it will be noted that the parts most strongly contracted or folded are those which fail to produce tentacles. In Fig. 9 both halves of the piece become slightly distended and tentacles appear on both except near the longitudinal cut where the edges are strongly inrolled. In Fig. 10 only the right side of the piece is capable of retaining water under pressure and only here do tentacles appear. The same is true of Fig. 12, while Fig 13 resembles Fig. 9 in that both sides become more or less distended. Figs. 14 and 15 show an interesting case in which the longitudinal cut surfaces have become greatly shortened and the mesenteries bent at an acute angle: these mesenteries are so closely approximated to each other that little or no water can enter between them and we find the tentacles corresponding to these chambers either minute and delayed in regeneration or absent. In Figs. 16 and 17 a case is shown in which closure was long delayed and the folds and wrinkles about the oral end were present for so a long a time that when the piece finally did close and become distended there were marked irregularities in the size of different intermesenterial chambers and consequently marked differences in rate

of growth and size of tentacles. The larger tentacles belong to those chambers which happened to be more or less widely open during the period of collapse. Some of the tentacles did not appear at all until complete closure and distension had occurred. In all cases after closure and distension the irregularities were gradually reduced until an approximation to the typical condition was attained. It seems scarcely necessary to multiply details further. My observations and experiments have led me to believe that in every case of this kind it can be shown that absence of tentacles or delay in their appearance is correlated with contraction, folding, etc., which may prevent the occurrence of circulatory currents in the intermesenterial chambers or reduce their force and volume.

The form of the marginal tentacles in open pieces is usually markedly different from that of typical tentacles, the former being blunt and rounded in form, often almost sac-like, instead of slender and tapering. A comparison of Figs. 2 and 4 with Figs. 3 and 5 affords an illustration of these differences; Fig. 16 represents an extreme case of this kind. In all such cases the blunt tentacles increase in length and soon acquire an almost or quite normal form after closure occurs. This peculiar form of the tentacles in open pieces may be regarded as due indirectly to the reduced size of the margin of the disc in these more or less collapsed pieces (cf. Figs. 2 and 4 with 3 and 5). Since the margin of the disc is reduced the opening into the tentacle from the intermesenterial chamber is smaller than in distended pieces, consequently the movement of water through it is impeded. Thus the local pressure exerted by the circulatory currents must be to a large extent eliminated within the tentacles, or at any rate insufficient to cause continued increase in their length. The general internal pressure, however, must be the same in the tentacular cavity as in the mesenterial chambers and it is possible that this has some effect upon the thin new tissue of the tentacles in causing enlargement in all directions.

It is possible also that the form of the tentacle may be affected to a greater or less extent by the rate of growth. In the open pieces the rate of growth is always slower than in closed pieces in consequence of the lower pressure in the former. Material

which is employed in closed pieces in producing increase in length of the tentacle, may, in the absence of the usual powerful stimuli to longitudinal growth, be used in regions where stimuli are less powerful and so in consequence of the general pressure in all directions bring about an atypical increase in the transverse diameter of the tentacle. According to this view the stimulus to longitudinal growth in closed pieces is so much stronger than the others that growth occurs almost wholly in this direction; this stimulus being reduced others become more effective. The reactions of various forms, both growth-reactions and motor-reactions, in response to stimuli might be cited in support of this view, for it is well known that in many cases the predominant stimulus determines the reaction.

Attention has already been called to the fact that in the open pieces with delayed tentacle-regeneration the tentacles usually acquire an arrangement in two or three rows much earlier than in the closed pieces with typical regeneration. I believe that this difference is due to the smaller circumference of the disc and the rounded, more or less sac-like form of the tentacles in open pieces. It is clear that the smaller the disc the fewer the tentacles which can be borne in a single row upon its margin; and when the tentacles themselves are less slender on pieces with the smaller discs than on the typical pieces, it follows that the crowding of some of them out of the single row must occur all the earlier. Figs. 2 and 4, as compared with Figs. 3 and 5, are good examples of this difference. In the closed, typical pieces, where the disc begins to expand as soon as regeneration begins, the tentacles are found mostly in a single row until a comparatively late stage of regeneration, when they are gradually forced into two or three rows. In the open pieces, on the other hand, the blunt, sac-like tentacles often form two more or less complete rows a short time after their first appearance. This difference between open and closed pieces, which can scarcely be explained on any other basis, affords very strong evidence in favor of the view that the arrangement of tentacles in the normal animal in about three rows is the result of mutual pressure. It is possible that slight differences in the circulatory currents, themselves due to differences in lengths of mesenteries or other structural or

functional features may determine the order in which the tentacles are crowded out of the single row, and thus more or less exactly the definitive mutual positions of the tentacles. Thus far, however, I have been unable to discover any such differences.

The small size of the disc in open pieces as compared with closed pieces has been mentioned frequently in the above discussion. Comparison of Figs. 2 and 4 with Figs. 3 and 5 shows the marked difference. In closed pieces the disk soon acquires greater diameter than any other part of the body while in open pieces, its diameter is usually considerably less, and never much greater than that of the more or less completely collapsed body. This difference, like the other noted, can scarcely be due to anything but differences in internal pressure. In the regenerating piece growth of new tissue at the oral end occurs at the cut surfaces and on the tentacular ridge, and the distance between these two regions of growth being slight, the intervening tissue soon becomes involved in the changes. These changes consist, as was noted in my first paper (Child, '03*a*), of reduction in thickness of the body-wall and the production of new tissue. The result is that the whole oral end of the piece becomes covered with thin new tissue, which reacts much more readily to pressure than the old thick tissue of the body-wall aboral to it. The internal pressure, although no greater here than elsewhere in the body, must cause more rapid increase in size here since the tissue is much less capable of resisting tension. If we take into consideration the circulatory currents which strike the body-wall around the whole margin of the disc we have an additional factor causing growth of the disc in the marginal portions. On the oral side of this region is the area of thin tissue composing the more central portions of the disc, on the aboral side the thick body-wall. If the former responds more readily to stimuli than the latter, which appears to be the case, it is possible to understand why the disc spreads laterally and acquires a greater diameter than the other parts of the body. At first glance it might appear that if the growth of the disc were due to the pressure of water it should grow only in the oral direction, but I think that the presence of the mesenteries and the different conditions of the new tissues on the two sides of the region of maximum pressure

is sufficient to account for the fact that a spreading of the disc as well as growth in the oral direction occurs. The disc is never flat, but always somewhat funnel-shaped, indicating that the margins are the regions of most rapid growth. It is possible that actual invagination of the ectoderm at the center of the disc to form the œsophagus does not occur, but that the surrounding parts are pushed orally and peripherally by the internal pressure, leaving this portion behind, as it were, since it is firmly attached to the mesenterial margins. Goette ('98) states that the development of the œsophagus in the young *Cerianthus* occurs in this manner, though he does not express himself regarding causes.

Although the above suggestions have been made in more or less positive form, since that was more brief and convenient, I desire to state once more that I am well aware of the hypothetical character of many of them. That the general internal water-pressure does play an important part in the regeneration and reëstablishment of the characteristic form in *Cerianthus* I believe the experiments already described have demonstrated. As regards the details, however, and especially as regards the effect of local pressure due to circulatory currents extended experiment is necessary before final conclusions are possible. These suggestions I have made in the hope that they may aid both in making clear the interpretation which seems to me possible and in pointing the way for future experimentation. In later papers I shall bring forward additional evidence upon the problem.

Summary.

1. In pieces which are kept open or opened repeatedly at the aboral end the appearance and growth of the tentacles, the growth of the disc, and in fact all regenerative phenomena at the oral end are delayed. The marginal tentacles which appear under these conditions are short, blunt, and more or less sac-like in form. Labial tentacles rarely appear at all until closure and distension has occurred.

2. In pieces cut in such manner that closure is prevented or delayed retardation of regeneration occurs as in the cases just mentioned.

3. In all pieces in which closure is delayed by any means the process of regeneration resumes its typical course after closure and distention are permitted to occur.

4. It is believed that these experiments demonstrate the important influence of the general internal water-pressure upon regeneration in *Cerianthus*. Complete elimination of internal pressure was impossible with the methods employed, therefore complete inhibition of regeneration cannot be expected in any case.

5. The retardation in regeneration of the tentacles and expansion of the disc may perhaps be due essentially to the decreased force and volume of the intermesenterial circulatory currents in open as compared with closed pieces.

6. Irregularities, such as local absence of tentacles, differences in time of appearance and local differences in size may be due to local differences in the circulatory currents which in turn are the result of local foldings, inrollings, or contractions of the body-wall.

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
October, 1903.

BIBLIOGRAPHY.

Child, C. M.

'03a Form-Regulation in Cerianthus, I. The Typical Course of Regeneration. Biol. Bull., Vol. V., No. 5, 1903.

'03b Form-Regulation in Cerianthus, II. The Effect of Position, Size, and other Factors upon Regeneration. Biol. Bull., Vol. V., No. 6; Vol. VI., No. 1, 1903.

'04a Form-Regulation in Cerianthus, III. The Initiation of Regeneration. Biol. Bull., Vol. VI., No. 2, 1904.

'04b Form-Regulation in Cerianthus, IV. The Rôle of Water-pressure in Regeneration. Biol. Bull., Vol. VI., No. 6, 1904.

Goette, A.

'98 Einiges uber die Entwickelung der Scyphopolypen. Zeitschr. f. wiss. Zool., Bd., LXIII., 1898.

CLOSURE OF LONGITUDINALLY SPLIT TUBULARIAN STEMS.

ALICE M. BORING.

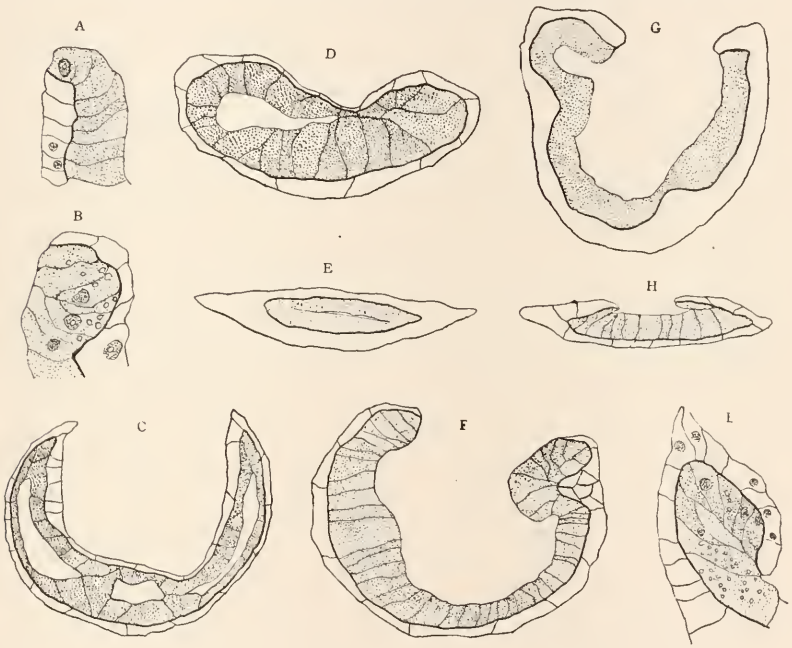
My work was carried on under the direction of Professor T. H. Morgan and Dr. N. M. Stevens, for whose suggestions and criticism I am deeply indebted.

The rapid closure of the cut edges of pieces of the stem of *Tubularia* which have been split longitudinally, has been studied by Morgan and by Godlewski. The latter has published the results of his observations in two recent papers. Godlewski worked with *Tubularia mesembryanthemum*, found at Naples, while my own work has been on *Tubularia crocea*, from Wood's Hole, Mass. The method of closing in this form appears to differ in a number of points from that of *Tubularia mesembryanthemum* described by Godlewski. Material that had been collected by Professor Morgan during the summer was sectioned and studied. The conclusions drawn from this work were then verified on fresh material, of which sections also were made. The study of the living material, however, is not very satisfactory, as it is possible to see so little of what is going on. The circulation of the fluid inside of the open tubes and the gradual stretching across of the membrane, are all that can be seen clearly.

The splitting was done with a pair of fine scissors. The material was killed and fixed in corrosive acetic. Somewhere in the process of preserving, probably in the alcohols, the cœnosarc shrinks and loosens from the perisarc, which keeps its former size and shape. This makes it difficult to study their relation to each other in the closing process. The tissues of the cœnosarc are well preserved by the corrosive acetic solution and can be studied easily. The sections were stained with Delafield's hæmatoxylin, followed by picric alcohol, according to the method of Stevens ('01). The picric alcohol stains the granules in the endoderm cells bright yellow, and thus makes the difference between ectoderm and endoderm cells distinct.

Method of Closure.

As the result of the splitting, the stems were divided into part-cylinders of various sizes, some almost complete, others with a mere strand of cœnosarc; while in some, an oblique split produced pieces nearly complete at one end and very small at the other. The method of closure seems to be independent of the size of the piece, the same general process being followed in all pieces. The cut leaves a free edge of both ectoderm and endoderm. In sections of pieces which had been killed five, ten or fifteen minutes after the splitting, the endoderm cells had rounded



up over the ectoderm, probably as a result of disturbing the osmotic pressures (Fig. A). The first step towards closure of the tube is the pushing of the ectoderm over the endoderm (Fig. B). Whether this is accompanied by any activity on the part of the endoderm cannot be stated definitely, but it seems probable from some sections and from what occurs in later stages, that the endoderm cells change their shape, their outer ends next the ectoderm becoming broader and the inner ends becoming pointed,

the cells thus taking on a triangular rather than a rectangular shape in section. This is shown in Fig. D. Some sections, however, do not show this at all, and we must conclude, that in some cases the endoderm lags a little behind the ectoderm.

During the subsequent process of closure, the ectoderm and the endoderm stretch across from both sides till they meet and form a two-layered membrane which closes in the digestive tract again. In this process of closing the two layers keep nearly even, but the ectoderm sometimes is a little farther advanced than the endoderm and curves around over its free end. The closing membrane does not arch outwards so as to complete the cylindrical form, nor does it grow straight across, which would be the shortest distance between the sides, but it is nearly always concave (Figs. C and D). In pieces which are much less than a half-cylinder, there is little chance for the membrane to bend in, and it lies close to the old wall (Fig. E). Also in cases where there is a large endodermal ridge, the bending in is either prevented or lessened.

The position of the endodermal ridges and their relation to the closure of the stem must now be considered. Godlewski has not taken these ridges into account, although one or two of his figures seem to indicate their presence. These ridges are found throughout the stem and cannot be ignored. Their presence causes some variations in the beginning of the process of closing and introduces certain conditions which might be easily misinterpreted. If the cut extends along the side of a ridge, or through it, the appearance in section of such a piece is very different from a section in which the ridge is not near the cut edge. There is a thick mass of cells at the edge, appearing somewhat like the beginning of a closing membrane (Fig. F). This mass is chiefly endoderm cells which contain many yellow-stained granules. If only a little of the ridge has been cut off, it is easily identified as a ridge by the thickening of ectoderm outside of it containing numerous dark stained nuclei; otherwise, identification is possible by tracing back through sections to a place where the cut was less deep and the ridge still intact. The ectoderm stretches over the cut endoderm until the two layers are even and then the two stretch across together and meet to form

a complete membrane. If the cut edge extends just above a ridge, when the membrane begins to stretch across, a cavity is left between the closing membrane and the ridge, which in some cases looks like a canal within the edge of the closing membrane, such as Godlewski describes (Fig. G). It may be that his canals are formed in this way and his figures might be so interpreted. In the sections of *Tubularia crocea*, I have found no canals formed by the disintegration of endodermal cells in the edge of the closing membrane. In the living material, no circulation has been observed within the edges of the closing membrane indicating the presence of canals, yet the circulation in the bottom of the open cylinder was easily seen, so that the circulation is not something peculiar to the canals of Godlewski.

The pieces may be cut off in such a way that a ridge is left in the middle of the half-cylinders. Sometimes it appears as though the growing edge of one side joined such a ridge, but this appearance was seen in but a few consecutive sections, showing that it would not have interfered with natural closing, in further development. If the piece is rather small, or the ridge large, the closing membrane is prevented from bending in as much as usual. But this middle ridge makes still greater complications in small pieces, as here it may fill up the cavity formed by the closing membrane, and cause an appearance of solidity.

In many of the smaller pieces, the sections, at first sight, look solid as though the endoderm cells had remained inactive, and lay in the same position that they were in when the piece was cut. It looks as though the ectoderm has grown over this solid mass, thus making a solid core of endoderm with a thin covering of ectoderm as Godlewski describes. But it is natural to expect that if the endoderm cells lag only a little behind the ectoderm cells in the closure of a large piece of a tubularian stem, they would do the same in a small piece of the same stem. If the work had been entirely with large and small pieces coming from different stems or different portions of the same stem, this expectation might not be justifiable, but the pieces used were obliquely split, large at one end and small at the other, the conditions for both ends thus being nearly alike. A contraction, if the closing is due to this, would be along the entire free edge regardless of

the size of the piece or the part of the tube, and if the endoderm and ectoderm both take part at one end, it is reasonable to expect that they would along the entire edge. With this idea in mind, the apparently solid closures were studied in order to see if there were not two layers of endoderm. The pieces were traced back through the series of sections to the place where less had been cut off in order to locate the ridges and in many pieces, a ridge was found included in the small piece. In some very small pieces, not including any ridge, it was found that the edges, both ectoderm and endoderm, had met to enclose a small but distinct cavity. Fig. H shows the section just before the edges meet. In other small pieces with no ridges the higher powers of the microscope showed two distinct layers of endoderm, indicating that the closure had been the same, but the piece was so flat and the angle turned by the closing membrane was so sharp that the cavity between the old and new walls was flattened out into a mere crack and the endoderm of the closing membrane practically touched the endoderm of the old wall (Fig. E). The presence of a ridge would cause the obliteration of the cavity in a piece otherwise large enough to leave a distinct cavity in closing. Fig. D shows this on the right side. The ridge is further toward the right, and consequently that side has no cavity. This section shows a circular arrangement of the endoderm cells at the point of turning, indicating a compression at their inner ends that would justify the statement that the layer of endoderm has been drawn around with the ectoderm.

In apparent contradiction to the above statement that only small pieces, or pieces including a ridge, close in solid, obscuring the cavity, a few sections complying with neither of these conditions show the ectoderm beginning to grow over the endoderm and leaving no cavity. But here, as in the small pieces there are two layers of endoderm in all cases where the ectoderm has overgrown, showing that the endoderm is following the ectoderm closely, and that a two-layered plate is really being formed even if obscured by the pressure of the two walls together (Fig. I). What the further development of such pieces would be, has not been definitely determined, but it is not likely that they close in to make a solid piece, for among the completely closed solid

pieces, there were none so large, unless the stem was thicker, or a ridge intervened.

Whatever the cause of closure may be, whether some kind of contraction or not, the fact can clearly be seen from the study of sections that cell-division has little or no part in the formation of the closing membrane; the new tube has practically the same number of cells as the open, part-cylinder. Mitotic figures are occasionally found, but not more than might have occurred in a normal tubularian, and in any case they were not frequent enough to indicate that mitosis is a factor in forming the closing membrane.

Bryn Mawr,
May, 1904.

REGENERATION IN THE PLANARIAN PHAGOCATA GRACILIS.

T. H. MORGAN AND ALICE E. SCHIEDT.

Phagocata gracilis differs from other planarians in having a number of pharynges in a common pharyngeal chamber. There is a large median anterior pharynx, and 12 or 14 lateral pharynges arranged along the sides of the chamber. The object of the following study was to determine the sequence in the regeneration of the pharynges in pieces from different regions of the body. The particular point that we had in view in beginning the work was to examine the origin of the new pharynges in pieces cut posterior to the region of the old pharynges. It has been shown for other planarians that pieces from this region of the body produce a new pharynx at or near the anterior end, and the question arose as to what would occur in a form like *Phagocata* having several pharynges. Would the median pharynx first appear and new ones be added behind this one? This would indicate that a growing region was also present *behind* the first pharynx, while in other planarians the growing region in pieces of this kind appears to be mainly anterior to the new pharynx. It seemed not improbable that a single anterior pharynx alone might develop and produce a mono-pharyngeal worm! Other similar questions arose in connection with the regeneration of new lateral pharynges in more anterior pieces.

The regeneration of the pharynges, in cross-pieces from the following regions of the body, was studied:

A. Worm cut transversely into five pieces.

1. Head-piece without any pharynges.
2. Median piece with old median anterior pharynx.
3. Middle piece with several lateral pharynges.
4. Posterior piece with posterior pair or pairs of lateral pharynges.
5. Tail piece without any pharynges.

B. Worm cut transversely into ten pieces.

C. Worm cut transversely into three regions: (i.) head, (ii.) region of pharynges, (iii.) tail piece. These three pieces were again cut transversely into as small pieces as possible.

An examination of the position of the new pharynges in the different pieces showed that in head-pieces the development occurred in the posterior part of the piece. In middle pieces new pharynges were regenerated at both ends; and in tail-pieces a new median pharynx appeared in the anterior part and new lateral ones were later added behind.

The first series (A) of preparations (sections) were of worms cut into five pieces and killed ten days later.

Five head-pieces: (i.) has a large central pharynx, four lateral pharynges, three on the left, one on the right.

(ii.) A central and three lateral pharynges are formed.

(iii.) A central and two lateral pharynges are regenerated.

(iv.) Four pharynges are formed; the largest is probably the median pharynx, but this is not certain because the piece had a peculiar shape, due to unequal cutting.

(v.) A central and four lateral pharynges are formed.

Five median pieces with the old central pharynx present.

(i.) The old central pharynx appears and five new lateral ones.

(ii.) An old central pharynx and, owing to imperfect cutting, old lateral ones are also found; three new lateral pharynges have regenerated.

(iii.) An old central pharynx and many new lateral ones are present.

(iv.) No old pharynx is found in this piece; a new central and three new lateral ones are formed.

(v.) An old central pharynx and possibly two or three lateral ones are left. There are several new lateral ones.

Five middle pieces. (i.) shows a new central pharynx and lateral ones anterior and posterior — the old lateral ones occupying the middle of the piece.

(ii.), (iii.), (iv.), and (v.) show a similar regeneration.

Of the five posterior pieces (i.), (iii.), and (v.) were cut too far back, and therefore only new pharynges are found. There are present a central and several lateral ones.

(ii.) and (iv.) have old lateral pharynges, and a new central pharynx and new lateral ones both anterior and posterior.

All the tail pieces show a new central pharynx and from two to five new lateral pharynges.

The second set of worms were cut into five similar pieces and killed at the same time as the first set.

Of the three head pieces (i.) and (iii.) show a new central and four new lateral pharynges.

(ii.) shows a central and three new lateral pharynges.

The median pieces all have a new central pharynx growing probably from the base of the old one. They all have several new lateral pharynges ; and worms (ii.) and (iii.) have two lateral old pharynges.

Each of the middle pieces shows many old lateral pharynges. (i.) shows one new lateral pharynx in the posterior end ; (ii.) shows two new anterior and one new posterior pharynx ; (iii.) and (iv.) show a new central pharynx and lateral ones anterior and posterior.

The posterior pieces (ii.) and (iii.) show a new central and new lateral pharynx in the anterior part, old lateral ones in the middle, and new lateral ones posteriorly.[1] (ii.) shows only a new central pharynx ; the rest are old. Of the tail-pieces, (i.) has a new central and five new lateral pharynges. In (ii.) no new pharynges have regenerated.

In (iii.) a new central and two lateral pharynges have developed.

The third set, cut into five pieces, were killed twelve days after the worms were cut.

The three head-pieces show a new central and four new lateral pharynges.

Of the median pieces (i.) and (iii.) show an old central and three new lateral pharynges. (ii.) has regenerated two new lateral ones posteriorly ; the four others, including the central one, are probably old ones.

The new central and several old lateral pharynges are present in the middle pieces ; new lateral ones are developed at both ends of the pieces.

Of the posterior pieces (i.) has one old and two new pharynges ; the central one is not yet regenerated.

[1] In this case it is probable that the cut removed some of the old posterior pharynges.

(ii.) has no old pharynges; but two new ones, one of which is the central pharynx. (iii.) has no old lateral pharynges in the posterior part, three new anterior ones, including a new central pharynx. Each of the tail-pieces has one new pharynx; (ii.) has also one lateral pharynx-bud.

In another series (B) the planarians, cut into ten pieces, were kept ten days before being killed.

(*a*) The head-pieces died with but one exception. In this a pharynx cavity is formed with, possibly, a pharynx-bud.

(*b*) The second pieces except worm (ii.) show new pharynges regenerated.

(iii.) and (iv.) have developed a new central and four new lateral pharynges.

(i.) has a central and three lateral pharynges.

(*c*) The third pieces all have a new central pharynx and from one to three new lateral ones. In one piece the anterior pharynx is probably the old one.

(*d*) The fourth pieces show new pharynges regenerated at at each end of the pharynx cavity, there being old ones in the middle. (i.) and (iii.) have a new central pharynx and several new lateral posterior ones, and several old ones in the middle.

(ii.) differs in having other new pharynges besides the central one in the anterior part.

(*e*) The fifth pieces are similar to the fourth, having new pharynges at both ends, old ones in the middle.

(*f*) Two of the sixth pieces have four new lateral pharynges posteriorly, the central one and the three anterior lateral ones being old.

(iii.) has a central and two lateral pharynges, probably all of them new.

(*g*) All of the seventh pieces have a new central pharynx, old ones in the middle and new ones towards the posterior end.

(*h*) One worm of the eighth set shows a condition similar to those of the seventh set. The other has only new pharynges, a central and two lateral ones.

(*i*) The ninth pieces have two or three new pharynges, (i.) has a central one, while in (ii.) no central pharynx has regenerated. No old ones are left.

(*j*) Two of the tenth pieces have not formed new pharynges, the third has a new central and two new lateral pharynges.

In another series (C) the planarians were cut into the three regions of the body — head, pharyngeal and tail parts and then each of these were cut into very small cross-pieces; they were kept nineteen days and then killed. In the head pieces a new central and from three to five new lateral pharynges have developed.

The pieces from the pharyngeal region give no results as the pharynges are too advanced for one to be able to determine which of the pharynges are old and which are new.

One of the tail pieces had no pharynges developed. The others had a central and from four to six new lateral pharynges regenerated.

The only other study that has been made of the regeneration of *Phagocata gracilis* is that of Lillie in 1901.[1] He showed that "the power of regeneration of this genus is equal to that of *Planaria*." He also pointed out that in cross-pieces several pharynges regenerate simultaneously, although the more anterior ones are the most advanced. Each pharynx develops at first in a separate cavity of its own and the cavities fuse together to form a common pharyngeal cavity. We have also observed this mode of development. Lillie found that in lateral pieces new pharynges first arise on the old side of the piece and the new ones on the opposite, regenerating side do not appear until a branch of the intestine has extended into this region. The new pharynges connect at one end with this branch.

Conclusions.

The regeneration of cross-pieces from different levels shows the following relations.

1. Pieces anterior to the old pharyngeal chamber produce in the new tissue at the posterior end a new median pharynx and lateral pharynges that increase in number from before backwards.

2. Pieces containing only the old median pharynx develop new lateral ones from the sides of the pharyngeal chamber.

[1] Lillie, F. R., "Notes on Regeneration and Regulation in Planarians," *Amer. Jour. Physiology*, VI., 1901.

3. Pieces containing only some of the lateral pharynges develop a new median pharynx at the anterior end of the pharyngeal chamber and new lateral pharynges both in front of and behind the old lateral pharynges.

4. Pieces cut behind the old pharyngeal chamber develop a new median anterior pharynx and new lateral ones are added from before backwards.

5. Old lateral pharynges do not appear ever to be substituted for the old median pharynx if this is removed.

ON THE SKELETON OF REGENERATED ANTERIOR LIMBS IN THE FROG.

ESTHER F. BYRNES.

While the skeletal structures of regenerated posterior limbs in the Anura have been found to be capable of almost complete restoration, I know of no similar studies on the anterior limbs. As the anterior limbs of Anurous batrachians appear relatively late in the life of the larvæ, some interest attaches to the power of regeneration possessed by these organs.

That the lost *regions* of the anterior limbs may be restored more or less completely has already been proven,[1] but how completely the *skeleton* can be restored within the various limb-regions it is the purpose of this article to show. The many less complete instances of regeneration which are of frequent occurrence after amputation of the anterior limb have been omitted from consideration.

A comparison of the regenerated skeletons seen in Figs. 2 and 3 with the normal skeleton figured in 1 shows that the skeletal structures are often less completely regenerated than might be expected from the outward appearance of the limb. In both cases there is a tendency not only to reduction in the number of digits in the regenerated hands, but also to a reduction in the number of phalanges in each digit.

The relatively greater length of the middle digit in both 2 and 3 marks it as undoubtedly the fourth digit, though in each case but three phalanges are present, while in the normal limb there are four. A similar reduction of parts likewise occurs in the fifth digit of both 2 and 3 which normally is also composed of four phalanges. In the case of the remaining digit in the regenerated hands, I am unable to determine whether it is the third or the second digit that has regenerated. In Fig. 2 the digit contains the normal number of phalanges, whether it be

[1] Byrnes, E. F., "Regeneration of the Anterior Limbs in the Tadpoles of Frogs." *Archiv für Entwickelungsmechanik der organismen.* Bd. XVIII., 2 Heft, 1904.

the third or the second. In Fig. 3 this digit has suffered a reduction in its parts along with the fourth and fifth, and in its relatively short length suggests the third digit, while the relatively greater length of the corresponding digit in 2 suggests that it may be the second. In both 2 and 3 the rudimentary pollyx is present. In the carpal region there is but little differentiation in

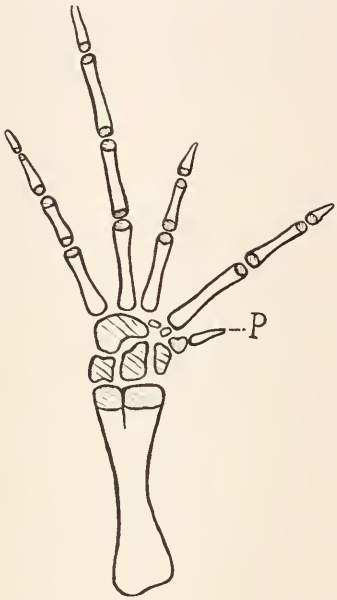


FIG. 1. The skeleton of a regenerated hand.

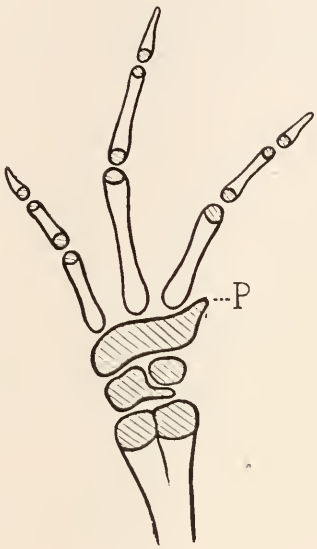


FIG. 2. A normal skeleton of an anterior forearm and hand. The striped are as are cartilaginous.

the regenerated limbs, there being but little ossification in the cartilage in 2, and no ossification whatever in the carpal region of 3.

A striking feature in the regenerated limbs is the constant appearance of the rudimentary pollyx. Sometimes this is represented by a separate cartilaginous structure as in Fig. 3, while in other cases it is a mere projection from the fused cartilaginous carpalia, as in Fig. 2. Even in hands that are greatly reduced and distorted after regeneration, the cartilaginous projection of the pollyx often persists, as in Fig. 4.

After regeneration the double nature of the radio-ulnare is invariably strongly marked by a deep groove showing the line of demarkation between the ulnar and the radial regions. There is no trace of where the amputation occurred, the regenerated end being perfectly normal in proportion, and showing complete continuity with the upper part of the bone.

While the frog larvæ were regenerating the imperfect limbs whose skeletal structures are shown in the text figures, similar experiments were made on some *Amblystoma* larvæ about three

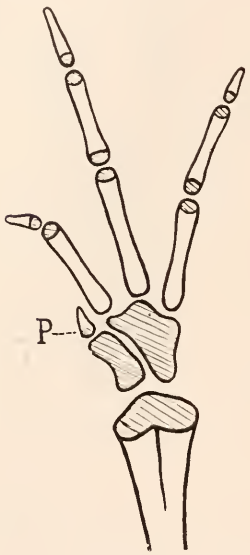


FIG. 3. The skeleton of a regenerated hand.

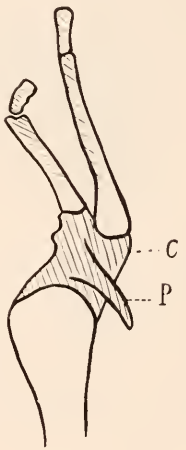


FIG. 4. The skeleton of a partly regenerated anterior hand. *P*, the pollyx ; *C*, the carpus.

months old. As soon as an *Amblystoma* had regenerated the lost limb, the regenerated limb was again amputated, so that toward the close of the experiments, the *Amblystomæ* were in possession of perfectly formed limbs, after having regenerated five in rapid succession. Each of the five limbs was not only normal in form and in the number of its parts, but it was also normal in *size*, the larvæ apparently having suffered no loss of power, even temporarily, to completely recover from the injury.

Early observers[1] have shown that in the Urodeles, the second digit is the first to appear in the process of regeneration, then the first, and subsequently the third and fourth digits. More recently, Ridewood has recorded a similar sequence in the development of the regenerated digits of the posterior limb of the midwife toad.

The concealed position of the anterior limb in the gill-chamber in the frog, makes the observation of sequence in the development of the digits difficult; but in the anterior limb, that region which appears first in the posterior limb of the toad and of the Urodele, is the one that in the frog is often wholly lacking.

Ridewood[2] has figured a case of regeneration in the skeleton of the posterior limb of the toad that shows that the limb is inherently capable of *complete* regeneration, and doubtless regeneration of the anterior limb may be much more complete in the frog than would appear from Figs. 2 and 3. Inasmuch, however, as these figures illustrate regeneration after amputation of the anterior limb when it was but very imperfectly formed, they indicate a marked tendency toward early loss of regenerative power.

BROOKLYN, NEW YORK,
March, 1904.

[1] Götte, A., "Ueber Entwickelung und Regeneration des Gliedmassenskelets der Molche." Leipzig, 1879.

Strasser, "Zur Entwickelung der Extremitätenknorpel bei Salamandern und Tritonen. *Morph. Jahrb.*, Bd. V., Leipzig, 1879.

[2] Ridewood, W. G., "On the Skeleton of Regenerated Limbs of the Midwife Toad (Alytes obstetricans)." *Proceedings of the Zoölogical Society of London*, Feb. 15, 1898.

OBSERVATIONS ON ANTS IN THEIR RELATION TO TEMPERATURE AND TO SUBMERGENCE.

ADELE M. FIELDE.

Although this paper finally treats of measures for the extermination of ants, the experiments [1] herein recorded were begun with a view to ascertaining the temperature preferred by ants and conducing to their rapid development in artificial nests.

The ants employed were *Lasius latipes*, yellow, translucent ants, from four to five millimeters long; *Stenamma fulvum*, brown ants, from six to seven millimeters long; *Camponotus pennsylvanicus*, black ants with dense integument, from eight to eighteen millimeters long. The ants were chosen with reference to color and size, a colony of *Camponotus* whose members varied exceedingly in stature having been previously captured.

A special nest was made, affording opportunity for the ants to choose temperature agreeable to them. It consisted of a copper [2] half-cylinder, three centimeters in diameter, in which the central portion of the interior, one meter or forty inches in length, was partitioned from the further extending ends, lined with white blotting paper apart from the copper, and imbedded, concave side upward, in cotton wadding which extended slightly over the edges of the half-cylinder at its sides. The half-cylinder was made level, so that gravity should not influence the behavior of the ants. The central portion was kept dry throughout, that the influence of humidity might be eliminated. It was covered with glass microscope-slides laid transversely, and topped with blotting paper so as to secure uniform darkness within. The layer of wadding between the edge of the copper and the glass roofing admitted sufficient air for the ants to breathe, and left no interstice for their exit.

At the right-hand end the bare copper extended beyond the supporting table and was heated by an alcohol flame. At the

[1] These experiments were made at the Marine Biological Laboratory, Woods Holl, Mass., in June, 1904.

[2] Material suggested to me by Dr. G. H. Parker.

left-hand end the bare copper was surrounded by ice. When all was established, the interior surface of the nest, tested by removing a slide and covering the aperture with a wad of cotton through which passed a centigrade thermometer, showed a range of temperature from 10° C. or 14° F. at the left-hand end to 60° C. or 140° F. at the right-hand end. The air was usually a degree or two lower than the enclosing surface that warmed it.

I then separately introduced into this nest different groups of the above-named genera, workers, larvæ and pupæ, in each case leaving the group to collect its scattered young at whatever portion of the nest the workers should themselves select. With great unanimity the different groups chose a place in the nest where the temperature was from 24° to 27° C. or 76° to 82° F., the temperature being taken from the surface against which the ants and young rested.

This is the temperature at which I have observed greatest activity and reproductivity in my artificial nests. I have also, when the temperature of the air was somewhat below 22° C. or 70° F. repeatedly observed wild colonies of *Stenamma fulvum*, where the larvæ and pupæ had been carried outside the nest and laid among the grass stems for greater warmth, despite the daylight from which the ant-nurses commonly withdraw the young.

In 1901 I froze[1] *Stenamma fulvum*, queens, workers and young, for twenty-four hours, the thermometer going down to — 5° C. or 23° F. The ants were gradually thawed and all survived. The frozen eggs, larvæ and pupæ developed perfectly later on.

Below 15° C. or 60° F. the ants are noticeably sluggish. Increase of activity accompanies increase of temperature from freezing point, where they are wholly inert and apparently lifeless, up to a degree where they swoon from the intensity of the heat. In the copper nest the ants manifested discomfort or distress at any temperature above 30° C. or 86° F. At 35° C. or 96° F. the smallest ants swooned, and I could induce no ant to voluntarily move to a hotter portion of the nest, even when driven by sun-

[1] "A Study of an Ant," *Proceedings of the Academy of Natural Sciences of Philadelphia*, July, 1901, p. 441.

light or when in pursuit of scattered larvæ which are commonly sought with self-sacrificing assiduity.

When the largest ants were introduced into the rest where the temperature was between 40° C. and 50° C. or 105° F. and 122° F. they either succumbed to the heat and swooned upon the floor or else struggled away toward the cooler end of the nest. All ants that swooned and fell where the temperature was below 50° C. recovered their activity if soon removed to cool and humid quarters. Ants exposed for two minutes to a temperature of 49° C. revived after some hours and resumed their normal occupations.

But exposure to heat so great as 50° C. or 122° F. produces in the ant pathological conditions from which it does not recover if the exposure is sufficiently prolonged. Probably the protoplasm of the ant coagulates at 50° C., the time required for the coagulation of all the protoplasm depending on the size of the ant. The time is the same for ants of the same size and species, whether the heat be imparted through a dry or a wet medium.

In order to ascertain the time required for the heat to kill the ants, I adopted a method which produced no mechanical injury. Grasping one or more legs of the ant with padded forceps, I merged the ant in air or water heated to just 50° C. and held it there for the recorded number of seconds, then laid it upon a cool moist sponge, to remain under observation during the five ensuing days or longer. Every ant appeared to die as soon as it reached the heated medium.

The first sign of revival, a slight twitching of the legs, was sometimes given many hours or even a day or two after the exposure to the heat. That these throes were those of returning rather than of departing life was proven by the complete recovery of some of the ants after days of manifest illness, abstentation from food and indifference to environment. Many ants revived that never wholly recovered.

Whenever any ant gave evidence of life, I provided hygienic conditions and trained nursing. Recuperation was slow in proportion to the length of time the ant had been exposed to the heat. One *Stenamma* that had been exposed twenty seconds, gave the first sign of returning life forty-seven hours later, and

died within a day. Another *Stenamma* revived forty-three hours after the same exposure and lived at least seven days. One *Camponotus* gave the first sign of returning life fifty-seven hours after its exposure of one minute to the heat and was more than a week in convalescence.

Lasius latipes. — When exposed ten seconds to heat at 50° C. twenty-three per cent. revived. When exposed for fifteen seconds none revived.

Stenamma fulvum.—When exposed to a temperature of 50° C. for ten seconds, forty per cent. revived; when exposed for fifteen seconds, fourteen per cent. revived; when exposed for thirty seconds none revived.

Camponotus pennsylvanicus.—When exposed to temperature of 50° C. for thirty seconds, all revived; when exposed for one minute, fifty per cent. of those of the largest stature revived; when exposed for two minutes, none revived.

Throughout the experiments, the smaller ants succumbed under the shorter exposures, while the larger ants were those that survived the longer exposures; but a temperature of 50° C. sooner or later proved fatal to all.

Submergence in Cool or Cold Water.

That the ants were killed by the heat and not by asphyxiation, when the medium was water, was shown by the fact that the ants succumbed during a like period of exposure whether in water or in air equally heated. But I tested the effect of submergence in cool or cold water and found that the ants survived such submergence for comparatively immense periods.

When ants are submerged they struggle or crawl about for a few minutes only, after which they sink and appear to have died. Many groups of ants were submerged with the following results:

Lasius latipes: Group a. — Fifty per cent. revived after twenty-seven hours' submergence and were alive and well six days later. The revivals all occurred within two hours after the ants were removed from the water to a moist sponge.

Stenamma fulvum: Group b. — All revived after eighteen hours' submergence.

Group c. — Seventy-five per cent. revived after fifty-two hours' submergence, and were alive and alert two days later.

Group d. — Fifteen per cent. revived after seventy-two hours' submergence, the first sign of revival appearing six hours after removal from the water. These survivors lived several days, but never resumed their normal activities.

Group e. — None survived eighty-eight hours' submergence.

Camponotus pennsylvanicus: Group f. — Thirty-three per cent. revived after forty-eight hours' submergence, and fully recovered. The first sign of revival was given twenty hours after removal from the water, and recovery occupied many ensuing days. The ants were about fifteen millimeters in length.

Group g. — Fifty per cent. revived after seventy hours' submergence, the first signs of revival being given from twenty-one to thirty hours after removal from the water. Full recovery did not occur until after a lapse of many days: but twenty-five per cent. were returned alive to a nest, where they met and were met by their former comrades, with manifest pleasure.

Group h. — Only ants from fifteen to eighteen millimeters in length were employed. After a submergence of ninety-five hours, none revived.

Such results show the futility of ploughing up ant-nests and exposing the ants to spring rains as a means of exterminating these pests on the farm. They indicate for this object the application of heat to the nests, through any medium wet or dry, the degree of heat being no less than 50° C. for any ants, and the duration of the application being at least fifteen seconds for the smallest ants and two minutes for large ones. Lured by greater warmth, the ants assemble with their young at or near the surface of the ground early in summer, and they would be surely destroyed either by shoveling the nests, with their millions of developing young, into a hot portable oven, or by saturating the nests *in situ* with water duly heated. The ants that injure growing crops by pasturing the aphides, their "milch cows," upon the roots and stems, are small ones, and they would probably be killed by an application of water hot enough to destroy the ants without injury to the plant.

The great tenacity of life in the ants and the distress evinced by them during slow recovery or dissolution, should impel those undertaking their extermination to do the work speedily and effectually.

Vol. VII. *September, 1904.* *No. 4*

BIOLOGICAL BULLETIN

THE TEMPORAL ARCHES OF THE REPTILIA.

S. W. WILLISTON.

Students of herpetology are indebted to Professor Henry F. Osborn for a careful taxonomic and morphological study [1] of the extinct reptilia, from which he has concluded that the class is divisible into two distinct subclasses or phyla, which he has called the Synapsida and Diapsida. The writer has studied his paper with much interest and desires to acknowledge his indebtedness to it for many new ideas and stimulating suggestions, though he is forced to differ from the author in some of his conclusions. A full discussion of this paper is not possible at the present time, and the present communication, will, therefore, be restricted to a discussion of the relations of the bones of the temporal arches of the reptilia, relations which really lie at the basis of any classification.

I may mention, however, that I do not see my way clear to accept the term Synapsida proposed by Professor Osborn for the group of single barred reptiles, since this group really does not differ in any essential respect from the Synaptosauria (in the wider sense) of Fürbringer [2] and differs from the Synaptosauria of Cope, as most recently defined by him [3] chiefly in the inclusion of the Cotylosauria. But, I believe that Cope was right in separating the two groups, since he recognized, as does Osborn, the ancestral relations of the Cotylosauria to both the single and double-barred reptiles. If all other reptiles are derived from them, and

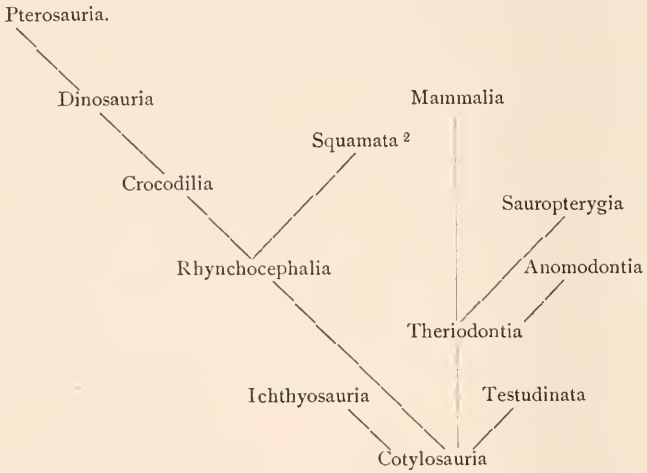
[1] "The Reptilian Subclasses, etc." *Mem. Amer. Mus. Nat. Hist.*, Vol. I., p. 451, 1903.

[2] *Jena Zeitschr.*, 1900.

[3] "The Crocodiles, Lizards and Snakes of North America," Rep. U. S. Nat. Mus., 1898, p. 159.

if the differences of these reptiles are sufficiently great to separate them into distinct orders, it would seem proper to distinguish the Cotylosauria from both the Synapsida and the Diapsida. Indeed Professor Osborn himself excludes them from union with either group in some of his definitions.

Furthermore, I cannot accept the conclusion as definitely proven, or even probable, that the reptiles are really diphyletic. The turtles seem, with much reason, to have had an independent origin from the cotylosaurs. My views in general of the pylogenetic relations of the orders of reptiles are pretty well expressed in the following diagram published by Cope in 1896.[1] It will be



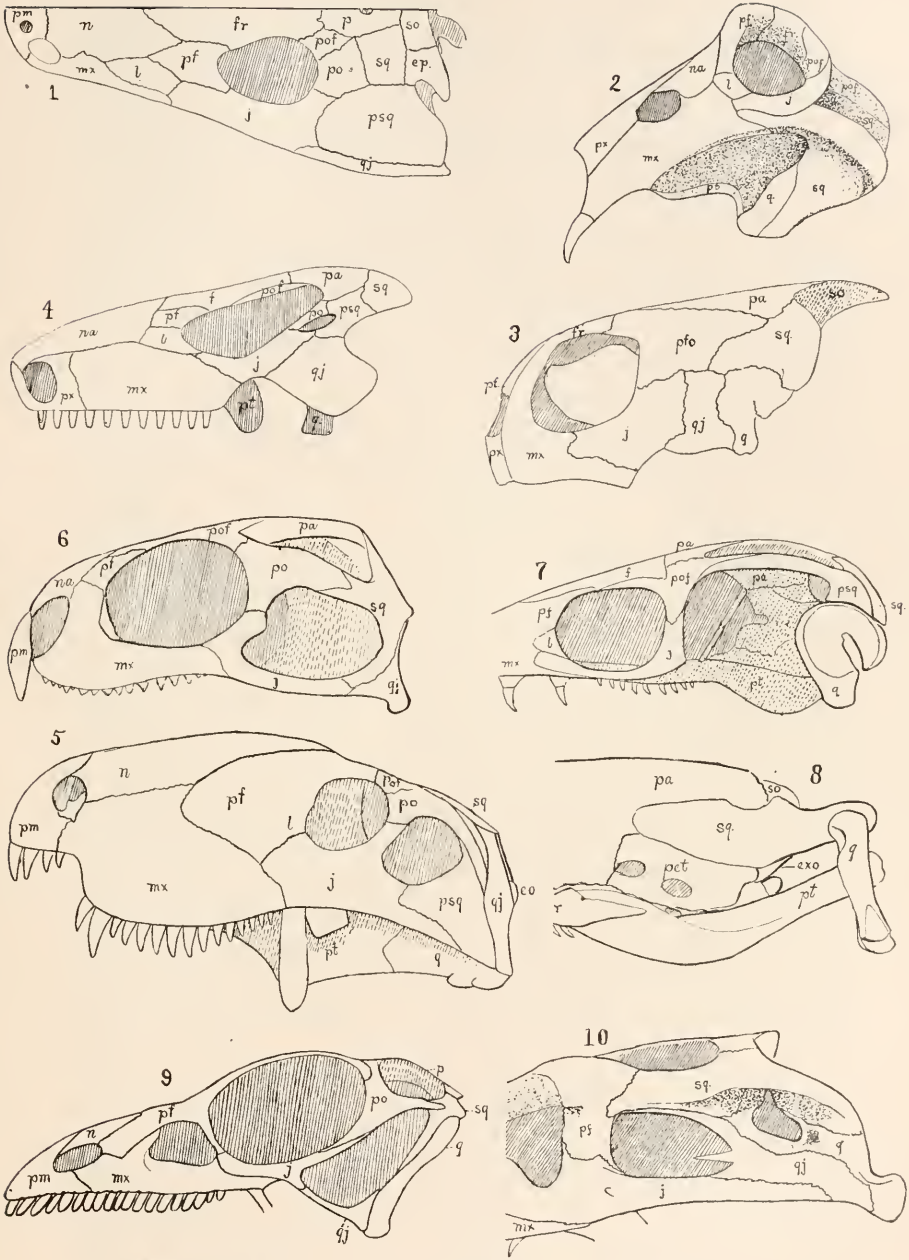
seen that, with the exception of the Ichthyosauria and Testudinata, the phylogeny scarcely differs from that of Professor Osborn, save in details. Osborn's characters are as follows:

Synapsida. — "A single large supratemporal fenestra; laterotemporal fenestra rudimentary or wanting. Bony elements of upper and lower arches not separated. Upper arch tending to degenerate first.

"Squamosals and prosquamosals large, expanded, early coalesced, forming a portion of the occiput, suturally covering the quadrate, secondarily entering the glenoid fossa.

[1] "Primary Factors of Organic Evolution," p. 115.

[2] "It is uncertain whether this order originated from the Theriodontia or the Rhynchocephalia." In the original it is derived from the Theriodontia.



1. *Mastodonsaurus*,
2. *Lystrosaurus*,
3. *Chelone*,
4. *Procolophon*,
5. *Dimetrodon*,
6. *Sphenodon*,
7. *Platecarpus*,
8. *Python*,
9. *Anchisaurus*,
10. *Gavialis*.

Diapsida.—" Large supra- and laterotemporal fenestræ ; laterotemporal fenestra sometimes secondarily closed. Bony elements of arch widely separated. Lower arch tending to degenerate first.

"Squamosals and prosquamosals often reduced, more generally separate, partially covering or withdrawn from the quadrate."

The closed or nonfenestrate condition of the temporal region has long been recognized as a primitive character in the reptilian skull. Cope, Baur and Seeley, and other authoritative writers have repeatedly called attention to the striking resemblances in the arrangement of the bones forming this region in some of the early reptiles and the Stegocephalia. Largely because of this resemblance some authors have urged the reptilian nature of the Stegocephalia, either uniting them with the reptiles as a branchiate division of the class, or placing them in a distinctive class, as " Proreptilia."

While these elements are so nearly alike in the stegocephs and cotylosaurs as to leave no doubt of their homologies, in the higher or more specialized reptiles the changes have been so great that the identification of some of the bones is doubtful. As a result of this uncertainty, many names have been proposed for the different temporal elements, aside from the postfrontal, postorbital and jugal, about which there has been no doubt or dispute. For the other four bones, however, I find the following names in use within recent years : epiotic, os tabulare, paroccipital plate, squamosal, prosquamosal, suprasquamosal, supratemporal, mastoid, supramastoid, supraquadrate, paraquadrate, zygomatic, quadratojugal, squamosal I., squamosal II. At the present time, the terms squamosal, prosquamosal, quadratojugal and epiotic seem to have most acceptance, though supratemporal is largely used for prosquamosal.

The temporal bones in the Stegocephalia and Cotylosauria are practically identical in number and arrangement (Figs. 1, 11). In both these forms it will be observed that the squamosal articulates broadly in front with the postorbital or postfrontal ; on the inner side with the parietal ; on the outer side with the prosquamosal, and sometimes the jugal. The prosquamosal unites broadly in front with the jugal ; on the outer side with the

quadratojugal. The quadratojugal has only a slight union with the jugal. The postfrontal joins by its whole length with the parietal. Now, no one will question but that this arrangement of these bones is the primitive one for the reptilia, and any rearrangement or readjustment must be a secondary result or specialization.

Among the higher forms, the nearest approach to this condition is seen in the testudinate skull (Fig. 3), in which the bony roof still remains unpierced; that is, there is no supra- or latero-temporal fenestra in such forms as *Chelone*, an undoubted primitive type of testudinate skull. The postorbital and postfrontal

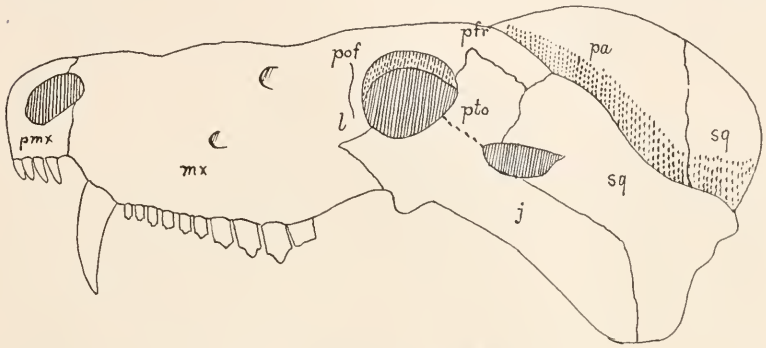


FIG. 14. *Cynognathus*, after Woodward.

are not distinct, or one or the other is absent. The prosquamosal has disappeared, or has become fused with some adjacent element. The quadratojugal has become greatly enlarged, separating the jugal from the quadrate, and articulating above with the squamosal — that is, it has taken the position of the prosquamosal in the *Pareiasaurus* skull. It is assumed that the prosquamosal, if present, is fused with the squamosal, but I fail to see any conclusive evidence of this; and it would seem more reasonable that, if the element is really present in the testudinate skull, it is fused with the quadratojugal, which has usurped its place and relations. Indeed the loss of the narrow bone on the outer side of the cotylosaurian skull would leave an arrangement pretty similar to that of *Chelone*, except that the quadrate is partly uncovered.

In the posterior part of the skull a vacuity has arisen, or, more

probably, was inherited from the cotylosaurs, between the parietal, paroccipital and squamosal, the posttemporal vacuity. By an emargination of the roof from behind, the squamosal may become separated from the parietal, and by an emargination from below,

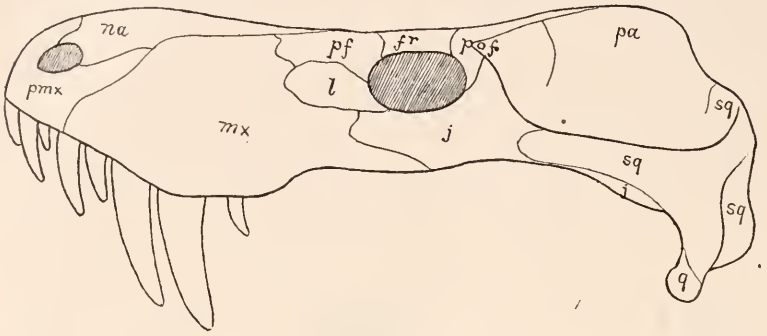


FIG. 12. *Lycosuchus*, after Broom.

from the postorbital, indeed, entirely isolating it in the arch. In such cases a large fossa is disclosed from above, which is sometimes, *though incorrectly*, called the supratemporal fossa. Baur some years[1] ago called attention to the fact that the turtles have no supratemporal fossa or fenestra. In fact, the temporal roof of the turtles throughout seems to be quite like that of the cotylosaurs, judging from Seeley's description:[2] "While the temporal vacuities are roofed over in *Pareiasaurus*, the roof is like the primitive roof of the genus *Chelone*, whereas in Professor Cope's figures of *Empedias* the skull appears to be closed behind as in Gorgonopsia." That the turtles ever had a supratemporal fenestra is quite out of the question. How, then, is it possible to derive them from the Anomodontia (*sens. lat.*), or from any known group of reptiles save the Cotylosauria? The entire absence of the quadratojugal bone, or any ossification corresponding to that bone in the Testudinata, in all the Anomodontia, Theriodontia, Placodontia and Therocephalia is exceedingly difficult to explain, if they are ancestral to the turtles. Has this bone reappeared, after its loss or close fusion with other bones? I am aware that many attempts have been made to show the relation-

[1] *Jour. Morph.*, iii., p. 472, 1889.
[2] *Phil. Trans.*, 1894, p. 1009.

ship of the turtles with the placodonts and plesiosaurs, but a study of these forms convinces me that whatever resemblances they may present can be accounted for by parallel development. Briefly, there are other characters in the skeleton that seem inconsistent with such a derivation from the Anomodontia, the intercentral attachment of the ribs, the presence of parial intercentra in the cervical vertebræ of some forms, etc. The conclusion seems irresistible to me that the Testudinata represent a distinct phylum of the reptilia, coördinate with the Synaptosauria or Synapsida.

By a sort of natural trephining of the skull-wall, a vacuity appeared between the squamosal and parietal, the squamosal still retaining its connection posteriorly with the parietal, and the

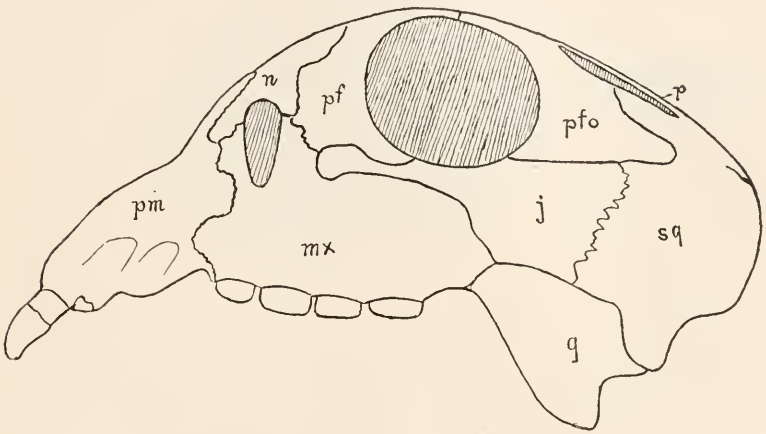


FIG. 13. *Placodus*, after Meyer.

postorbital taking a part in the separated bar thus formed. This is the condition in the Anomodontia and Sauropterygia, but never in the Testudinata. In the Anomodontia and Sauropterygia, concerning whose relationships there can be no doubt, the prosquamosals and quadratojugals have disappeared, leaving a single bar composed of the squamosal, postorbital and jugal. It is also assumed here that the prosquamosal and perphaps also the quadratojugal have become fused with the squamosal, but of this there is not a scintilla of evidence. A separate ossification, it is true, has been said to occur in some plesiosaurs by Owen,[1] which

[1] *Trans. Geolog. Soc. Lond.* (2), V., pt. iii., pl. XLV., 1840.

might be supposed to be the prosquamosal, but in the excellent skulls of four genera of these animals which I have examined there is positively no such separate element present, nor was there any recognizable prosquamosal present in the skulls described by Andrews. Nor has such an element ever been recognized in the Nothosauria. I am aware that Koken[1] has suspected the presence of such an element in one specimen of a nothosaur, but his evidence was very doubtful and has not been confirmed. I am confident that the true squamosal in these groups, as in the cotylosaurs and turtles, articulates directly with the post-

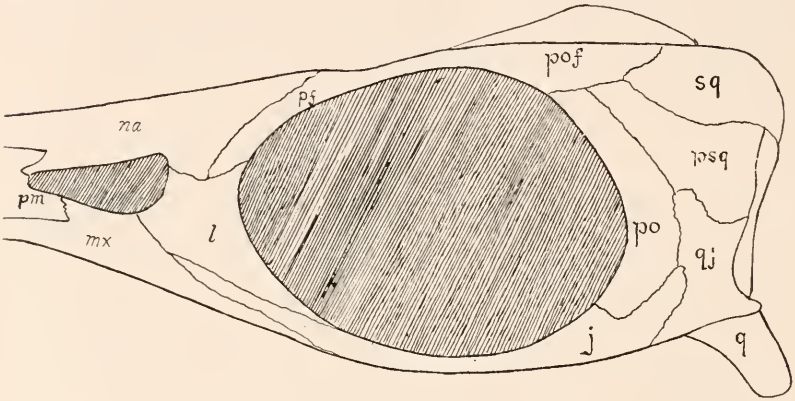


FIG. 17. *Ichthyosaurus*, after Owen.

orbital and jugal, without the intervention of any other element, and that the prosquamosal is wanting, not fused with adjoining bones; nor is there any certain evidence of the presence of the quadratojugal in any of these reptiles. In certain plesiosaurs I have found and figured what I believed to be a distinct ossification — a small, perhaps rudimentary one — that I took to be the quadratojugal. But I have never been able to trace it out, and no other observer has ever distinguished this element in the Sauropterygia. In the Anomodontia no such element has been certainly found, and, since we have the best of reason for believing that the anomodonts and theriodonts are closely related to the ancestors of both the nothosaurs and the plesiosaurs, we can hardly expect to find the bone distinct in these latter forms.

[1] *Zeitschr. Deutsch. Geolog. Gesellsch.*, XLV., 1893; p. 362.

Since the above was written, I have received the following statement from Dr. Broom, kindly sent in reply to a query from me: "No trace of either prosquamosal or quadratojugal has been found in any Anomodont or Theriodont. The large bone which supports the quadrate in *Dicynodon* and also forms so large a part of the temporal arch is all pure *squamosal*. The same is the case in the Therocephalia and Theriodontia. The upper part of the quadrate which rests on the squamosal has been thought by some to be the quadratojugal, but I am pretty confident that the whole is quadrate, and that no rudiment even of the quadratojugal is present."

From these facts we may conclude that the emargination of the single bar has removed all of the lower arch, and that all which remains corresponds to the upper arch of the diapsid reptiles, the squamoso-postorbito-jugal bar. Certainly until some form is discovered in which the prosquamosal and quadratojugal are definitely shown to be present as a part of the arch, the compound nature of the arch in the synapsid forms is hypothetical. One species of *Cynognathus* has been figured (Fig. 14) with a small fenestra between the squamosal and jugal. No other species of this genus possesses this opening, and its presence in this species, is, I believe, disputed by Dr. Broom. Even if it be present, it has no classificatory significance, since it must have been a parallel development in these animals; an abortive attempt, as it were, without phylogenetic significance. It is of interest, perhaps, if it really occurs in any of the anomodonts, as showing the tendency to perforation of a broad plate covered on both sides by muscles.

The question is properly asked: How has the mammalian zygoma arisen? The answer does not seem doubtful to me. The mammalian arch has the structure of that of *Lycosuchus* (Fig. 12), and is composed of the squamosal and jugal alone. This it seems to me, will be made apparent by the consideration of the arch in *Placodus* (Fig. 13), *Cynognathus* (Fig. 14), and *Lystrosaurus* (Fig. 2), in which the articulation of the squamosal is with both the postorbital and the jugal in about equal measure.[1]

[1] "I can find no difference in the character of the arch in the Anomodontia and Theriodontia except that in the Theriodont the malar bone has a greater external back-

In all the plesiosaurs (Fig. 16) the union of the squamosal with the postorbital has become much reduced, they merely touching each other and approaching the theriodont type, in which the postorbital has become wholly separated from the squamosal (Fig. 12).

The assumption that the prosquamosal or quadratojugal has thrust itself up between the squamosal and the postorbital is gratuitous, without evidence to support it. And, if they have not been thrust upward into this intercalary position, it must be assumed, if the bones are really present, that they form the lower part of the bar, parallel with the squamosal, intercepting the jugal from union with the squamosal. That the jugal may unite with the squamosal, even when the prosquamosal exists as

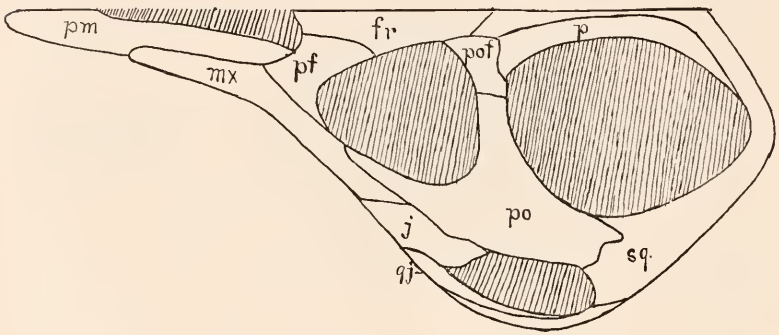


FIG. 15. *Stenometopon*, after Boulenger.

an independent ossification, is a fact, as is shown by the structure in the cotylosaur skull. Why then is it necessary to assume that these bones, or either of them, are present in the anomondonts and sauropterygians in a fused condition?

It is of course possible that the quadrate has also become a part of the mammalian arch, as has been urged by Dollo, Albrecht, Baur and others. The extraordinary development of the squamosal bone in the anomodonts and theriodonts has not only crowded out the prosquamosal and quadratojugal, but has also caused the absorption of the quadrate, enclosed between it and

ward development than is usually seen in the Cynodontia; but the difference between the groups is not due to any difference in the nature of the arches, but to a less development of the quadrate bone in the Theriodontia, which has resulted in a diminution or atrophy of the descending pedicle of the squamosal bone" (Seeley, *Phil. Trans.*, 1894, p. 997.)

the skull wall, the squamosal taking its function as an articulating bone for the lower jaw. I see no urgent reason for insisting that a vestige of it must yet be present in the eutherian skull, either as a part of the zygomatic arch, or in a totally different function as one of the ear bones. We are quite sure that some of the bones of the reptilian skull are not present in the mammalian skull, why may not the quadrate be one of them? There seems to be an idea that bones of the reptilian skull can only become lost by their union with contiguous bones, their ossific centers finally disappearing. But I am skeptical of this. We know that the quadratojugal is not present in the Squamata; that the 'prosquamosal' of the arched lizards is not present in

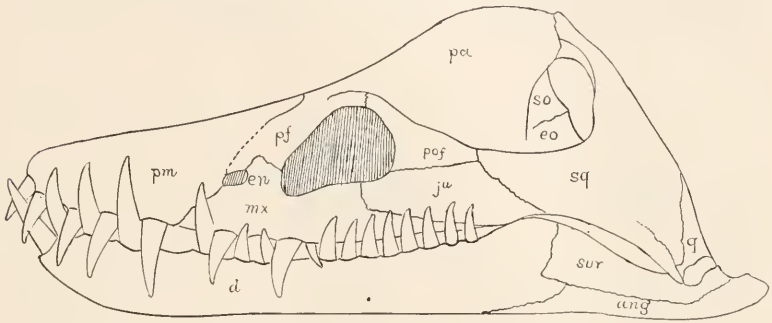


FIG. 16. *Cimoliasaurus*, original.

the amphisbænians or snakes; that the epipterygoid is also wholly wanting in some of the lizards, as well as other bones. Must we insist that their loss has always been by fusion and loss of ossific center? Must we insist that the lachrymal bone is always present in the turtles, snakes and *Sphenodon*; that the jugal is still a part of the postorbital or maxilla of the snakes; that the prevomers still remain as a part of some other bones in the eutherian mammals; that the splenial and coronoid still remain in the mammalian mandible?

Turning now to the double-barred or diapsid forms, it is a question which of the two vacuities appeared first, or whether they did not appear together. In *Ichthyosaurus* and *Aëtosaurus* we have a single vacuity, but the relations of both these forms are so evident with the early rhynchocephaloid reptiles that

there is perhaps good reason for the belief that the original lateral fenestra in the icthyosaurs has become closed up by the posterior obtrusion of the orbit, as was suggested by McGregor. That this was really the case, however, has by no means been proven. We can conceive of an early diapsid stem without lateral fenestration (as is indeed the case, in *Procolophon*), from which the ichthyosaurs might have been derived. Certainly the Ichthyosauria are the most primitive of reptiles, save the cotylosaurs, in so far as the lateral temporal region is concerned. The squamosal and postfrontal here form the outer boundary of the supratemporal vacuity, while the large prosquamosal is intercalated between the squamosal, postorbital and quadratojugal. If the ichthyosaurs originally had a latero-temporal fenestra it was situated, in all probability, above the prosquamosal. (Fig. 17.)

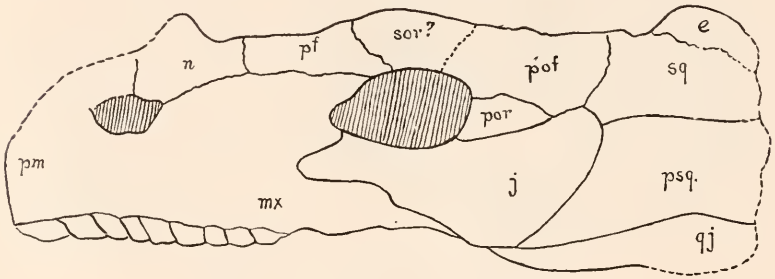


FIG. 11. *Pareiasaurus*, after Seeley.

Next to the ichthyosaurs, the most primitive of the diapsid types, as urged by Broom and Osborn, seems to be *Procolophon*. (Fig. 4). Here there is a peculiar arrangement of the bones of posterior region. A slender one by the side of the frontal and parietal, above the orbital opening, must be the postfrontal; while another bone articulating with the parietal and jugal, situated below and behind the eye cavity, is as certainly the postorbital. The orbit, we may conclude, has been extended backward, not by the closing up of the laterotemporal vacuity, but by the absorption of the postfrontal and postorbital, so as to come into contact with the parietal between them. But the orbit does not include the supratemporal vacuity, since neither the postorbital nor the squamosal helps form its outer boundary. There

is no evidence, in my opinion, that the large eye-opening has been formed by the coalescence of the supratemporal and orbital vacuities, but rather by the extension backward of the orbit, through the absorption of the postorbital bones. At the posterior part of the skull is seen an element which was believed by Seeley [1] to be the epiotic, a bone otherwise unknown, save possibly in some anomodonts, in the higher reptilia. Woodward, [2] however, identifies this bone as the squamosal, and the so-called squamosal in front of it he cails the prosquamosal. I do not feel at all sure which view is correct. If the two elements are really the epiotic and squamosal of the cotylosaur skull, I fail to understand how *Procolophon* could have stood in any very intimate relations with the early rhynchocephaloid reptiles, for they primitively had a prosquamosal. Professor Osborn states that all the elements of the cotylosaur skull are present in *Procolophon*, but neither his figure, nor Seeley's nor Broom's descriptions indicate the presence of all three bones, the epiotic, squamosal and prosquamosal — one of these elements seems certainly to be absent. Between the postorbital and the squamosal, there is a small vacuity shown in the original figure of this reptilian skull, the one reproduced here, which has been believed to be the laterotemporal fenestra. But, if I am correct, Dr. Broom denies the presence of this opening, and the figure he has been kind enough to send me shows no laterotemporal vacuity, in that position at least. Are we to suppose, such being the case, that, in addition to the absence of a supratemporal vacuity, the lateral opening also has been closed up secondarily? It seems to me that such reasoning savors a little too strongly of the ante-Baconian methods.

While *Procolophon* is shown by Broom, from certain evident peculiarities of the skull and skeleton to be more nearly related to the Rynchocephalia than to the Anomodontia, I fail to see any striking resemblances in the temporal region. It is a diapsid without temporal fenestræ.

Perhaps the most primitive known of the truly rhynchocephaloid type of reptiles, so far as the temporal region is concerned,

[1] *Phil. Trans.*, 1889, p. 271. "It rests by a squamous overlap upon the posterior border of the squamosal, and the external surface of the parietal."

[2] "Vertebrate Paleontology," p. 149, Fig. C.

is the Pelycosaurian *Dimetrodon* (Fig. 5) from the Permian of Texas, recently fully made known by Professor Case.[1] Here we have, in addition to the fully developed superior opening, a large fenestration below the squamoso-postorbital bar, and bounded below by the jugal and prosquamosal. The quadratojugal is relatively small, intercalated between the prosquamosal and the quadrate, and wholly separated from the jugal. *Paleohatteria* may possibly have a separated prosquamosal in the same position and with the same relations, but this fact, if fact it is, is yet to be determined. The Triassic *Hyperodapedon* and the allied *Stenometopon* (Fig. 15) have no separated prosquamosal. *Saphæosaurus*, from the Jurassic, more nearly ancestral, perhaps, to the modern *Sphenodon*, in all probability possesses a separated prosquamosal intercalated between the quadratojugal and the jugal, as in *Dimetrodon*. Finally in the living *Sphenodon*, notwithstanding its many primitive characters, the prosquamosal has utterly disappeared, even in the embryo, according to Howes and Swinnerton, though the squamosal is continued above the quadratojugal to unite with the jugal (Fig. 6). Here the quadratojugal articulates with the jugal, as in all the archosauria, the intervening bone having disappeared. Baur[2] believed that the prosquamosal was present in *Sphenodon* though early fused with the squamosal, a view which now is shown to be incorrect. The bone has been gone so long that it fails to make any impression upon the embryo.

From all of which evidence, *Dimetrodon*, *Saphæosaurus*, possibly *Paleohatteria*, and the conditions now existing in *Sphenodon*, the conclusion is irresistible that the laterotemporal vacuity was originally formed in the true diapsid reptiles not below, but above the prosquamosal; that this bone has nothing to do with the upper bar in these reptiles, which without exception was formed primitively by the squamosal and postorbital; secondarily by the squamosal, postorbital and jugal; finally in the theriodonts and mammals by the squamosal and jugal alone. The prosquamosal never forms the outer margin of the supratemporal fossa.

All this leads us to the consideration of the arch in the Squamata wherein the condition of things has caused no end of con-

[1] *Journal of Geology.*

[2] *Anat. Anzeiger*, X., p. 321, 1895.

troversy and differences of opinion, differences which are by no means yet settled. The single arch present (Fig. 7) has been variously considered to be the lower arch, the upper arch, or a compound of both arches. It is composed (when present) of three bones; an anterior one, the postorbital, extending backward to unite with a middle one; the middle one joining the postorbital in front, the posterior one and often the parietal behind, and more or less broadly articulating with the upper end of the quadrate; and a posterior element, joining the parietal internally, the middle element anteriorly, and united with the petrosal and exoccipital below, and usually also articulating with the head of the quadrate. The middle bone has been called the temporal, squamosal, quadratojugal, prosquamosal, supratemporal, zygomatic, supramastoid, paraquadrate; the posterior element, the mastoid, squamosal, supratemporal, supramastoid, and paroccipital. At present most writers, following Baur,[1] call the middle element the prosquamosal, and the posterior one the squamosal, though Woodward[2] applies the name squamosal to the middle element, and supratemporal (prosquamosal) to the posterior one.

It is now generally assumed that the Squamata have descended from the rhynchocephaloid reptiles. We have seen that in the early diapsid reptiles the prosquamosal is not articulated between the squamosal and the postorbital, but forms a part of the lower arch between the quadratojugal and the jugal. Here, however, it is assumed that the lower arch of the rhynchocephs has disappeared, that the quadratojugal is lost, but that the bone articulating with the quadratojugal between it and the jugal has been transferred to the upper arch, a position unknown in any other reptile, recent or extinct, to bound the outer part of the supratemporal vacuity. One could with as much reason call it the quadratojugal with former authors and with Baur[3] when he believed that the Squamata were not closely related to the rhynchocephalians, and that the arch of the Squamata was formed, not by the loss of the lower arch, but by the fusion and attenu-

[1] *Anat. Anzeiger*, X., p. 328, 1895.

[2] "Vertebrate Paleontology," p. 143, Fig. E; p. 192, Fig. A, 1898.

[3] *Journ. Morphology*, III., 473, 1889.

ation of a compound arch. One thing seems very probable, if this bone is the prosquamosal, then the Squamata have nothing to do with the rhynchocephaloids, but represent a separate and distinct phylum of their own. I prefer to call the bone articulating with the postorbital the squamosal, the bone which in all other reptiles articulates with the postorbital behind.

Of course, if this is the real squamosal, the posterior element cannot be a squamosal, though Koken [1] thought to solve the difficulty by calling the two bones squamosal I. and squamosal II. The history of the contention between Cope and Baur [2] as to the identity of this bone is too fresh in the minds of anatomists to need repeating here. Baur vigorously urged that the bone at the end of the suspensorium is the squamosal, but Baur never fully understood the relations of this bone in the mosasaurs, as is evidenced by his faulty description of it.[3] As Cope has repeatedly affirmed,[4] and as I have confirmed,[5] the so-called squamosal of the mosasaurs is intercalated between the exoccipital and petrosal, extending far inward nearly to the surface of the brain-case. It needs but a moment's consideration by any one familiar with the relations of the bone in these animals and in the mammals to be convinced that such remarkably different conditions cannot be those of the same bone. The inner part of the "squamosal" corresponds quite well with the outer part of the paroccipital, or opisthotic element, which was not found in the lizard embryo by Parker. Referring now to the figures of *Procolophon* and *Parciasaurus*, it will be seen that the outer part will correspond fairly well with the one called the epiotic. "In some of the genera of Stegocephalia the paroccipital is free from the exoccipital; in others (*Mastodonsaurus*) it is coössified with the exoccipital. The paroccipital is in relation to a dermal plate which is very improperly called the epiotic. I propose the name '*paroccipital plate*' for it." [6] It may be objected that the presence of an epiotic bone in the lizards is a far too primitive char-

[1] *Zeitsch. Deutsch. Geol. Gesellsch.*, XLV., p. 363, 1893.
[2] *Amer. Nat.*, 1895, 1896.
[3] *Jour. Morphology*, VII., p. 14, 1892.
[4] *Trans. Amer. Phil. Soc.*, XVII., p. 19, 1892.
[5] *Univ. Geol. Surv. Kans.*, IV., p. 121, 1898.
[6] Baur, *Journ. Morph.*, III., p. 469, 1889.

acter, but we are now quite certain that the lizards are an exceedingly old group, probably dating from the Permian, and that they have not a few very primitive characters, such as the presence of pterygoid teeth; perhaps the early forms, like those of the early crocodilia, will all be found to have amphicœlous vertebræ. However, whether or not this outer plate has early fused with the paroccipital beneath it, and has remained persistent in the lizards, I will not say, but I do believe that the bone corresponds to the paroccipital.

The archosaurian type of arches is one easily derivable from the Rhynchocephalia — a conjoined postfrontal and postorbital uniting posteriorly with the squamosal, to form an upper bar; and a quadratojugal intercalated between the jugal and quadrate to form the lower bar. The variations in the dinosaurs (Fig. 9) and the crocodiles (Fig. 10) are not great, and doubtless a like structure will be found in the pterosaurs when this part of their anatomy is better known than it is at the present time.

Returning now to the theses which headed this discussion, we see: That, in the Synapsida, neither the Cotylosauria nor the Testudinata have a large or any supratemporal vacuity; that, the cotylosaurs, if ancestral to the Synapsida, the Diapsida and the Testudinata, cannot be properly included into a subclass with any one of them; that the turtles could not have been derived from the Anomodontia, but apparently represent a distinct and independent branch from the Cotylosauria, or at the least from the primitive stem of the Synapsida before it had developed a supratemporal fenestra; that there is no evidence of a prosquamosal bone in any of the Synapsida (excluding the cotylosaurs), and little of the quadratojugal in the Anomodontia and Sauropterygia; that it was the elements of the lower, not the upper arch which became degenerate, leaving the mammalian zygoma to be composed of the squamosal and jugal only.

That, in the Diapsida, the prosquamosal is known to be present in but two forms; that it was an element of the lower, not the upper arch; that the arch of the lizards is the upper, not the lower one, and consequently does not contain the elements of that arch; that *Procolophon*, though a primitive diapsid, had

neither upper nor lower temporal fenestra, and probably never had.

These, it seems to me are legitimate conclusions from the evidence now available. But the evidence is yet meager, and all our present views may undergo material modification when more of the early reptiles from the Trias and Permian are known.

Explanation of Figures.

ep., epiotic.
exo., exoccipital.
fr., frontal.
j., jugal.
l., lachrymal.
mx., maxilla.
n. or *na.*, nasal.
p. or *pa.*, parietal.
pf., prefrontal.
pm., premaxilla.
pof. or *pfr.*, postfrontal.
po., or *pto.*, postorbital.
psq., prosquamosal.
pt., pterygoid.
q., quadrate.
qj., quadratojugal.
so., supraoccipital.
sq., squamosal.

In Fig. 4, *Procolophon*, the determination of the squamosal and prosquamosal is that of Woodward. According to the interpretation of Seeley, Broom and Osborn, *sq.* is the epiotic, *psq.* the squamosal. I am under obligations to Prof. Case for permission to use his figure of *Dimetrodon* in advance of publication by him.

FORM-REGULATION IN CERIANTHUS, VI.

Certain Special Cases of Regulation and their Relation to Internal Pressure.

C. M. CHILD.

In this paper are described certain regulative phenomena of interest both from a descriptive point of view and because they appear to offer further evidence upon the problem of the rôle of internal water-pressure in regulation. At present I can see no other satisfactory interpretation of these cases than that based upon internal pressure. In any case, however, they are interesting modifications of the typical course of regulation and as such deserve mention.

The Oral Regeneration of Oblique Pieces.

Pieces with oblique ends are obtained by sectioning the body at various angles to the principal axes as indicated in Fig. 1. Such pieces show a very marked difference in rapidity of regeneration on the different parts of the oblique cut surface, regeneration being most rapid on that part which represents the region nearest the oral end of the parent body and least rapid on the opposite side. This difference is due in part to the fact discussed in my second paper ('03*b*), viz., that the rapidity of regeneration always decreases with the increasing distance of the regenerating surface from the oral end of the original animal. But the difference in the rapidity of regeneration on the different parts of the oblique surface is considerably greater than that between control pieces with transverse cut surfaces at corresponding levels. It follows from this that some other factor in addition to the difference of level is concerned in the result.

Before proceeding to the description of experiments it should be said that the possible significance of oblique pieces was not fully recognized during the course of my experiments, therefore my study of such pieces was less extended than it would otherwise have been. The data are sufficient, however, to show

clearly the consequence of cuts of this kind. *C. solitarius* was used for those experiments.

Series 9.

September 12, 1902.— Three specimens of *C. solitarius* were cut obliquely at two different levels as indicated in Fig. 1; the pieces used for experiment in each case being the portions between the two oblique lines. These pieces possessed an oral and an aboral oblique surface. Unfortunately no control pieces with transverse cut surfaces were made but the records in my notes of a set of pieces which were cut at the same time and kept in the same aquaria will serve as controls. These pieces were prepared as



FIG. 1.

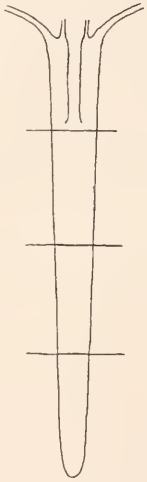


FIG. 2.

follows: after the removal of the oral end from two specimens by means of a transverse cut just aboral to the œsophagus the remaining portion of the body was cut into three equal pieces (Fig. 2). These pieces may be designated as *A*, *B*, and *C*, *A* being the most oral of the three. By comparison of Figs. 1 and 2 it is seen that the oral end of the control piece *A* is at almost the same level as the most oral portion of the oblique piece, the latter being slightly more oral, while the oral end of piece *B* corresponds very closely in level with the most aboral portion of the cut surface of the oblique pieces. In sectioning living animals so con-

tractile and changeable in form as *Cerianthus* the correspondence between the levels of cuts on different specimens is always only approximate. In this case the levels at which the oblique cuts were made differ more or less in the three specimens as well as the levels of the transverse cuts in the controls. Any control is therefore only approximate and the pieces used for this purpose here are as satisfactory in this respect as those employed in other experiments on *Cerianthus*.

In the control pieces *A* and *B* the rapidity of regeneration is that characteristic of the level of the body at which the oral ends of the pieces lie. Comparison of this rapidity with that of the extreme levels of the oblique cut surfaces will show whether the obliquity of the cut has any effect on the rapidity of regeneration.

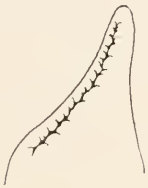


FIG. 3.

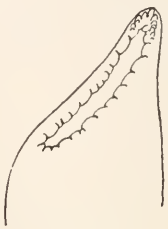


FIG. 4.

After the operation the collapsed oblique pieces acquired a form like Fig. 3. During the next day or two the cut edges rolled inward still more closely and the pointed oral portions became more or less bent over toward the cut surfaces. All became more or less flattened as they lay upon the flat glass bottom of the aquaria.

September 15. — 3 days after section. Experimental pieces: One piece with a few minute tentacle-buds at the uppermost portion of the oblique cut surface; others without tentacles.

Controls. All without tentacles.

September 17. — 5 days after section. Experimental pieces: All the pieces were completely or nearly closed orally by new tissue: the aboral ends were not completely closed by new tissue but were closely approximated and two of the pieces were slightly distended. Each of these pieces possessed a small group of minute marginal tentacle-buds at the extreme oral part of the

oblique surface (Fig. 4). No traces of tentacles were visible elsewhere. In the third piece the unpigmented tentacular ridge was visible at the most oral portion of the oblique surface but tentacle-buds had not yet appeared.

Controls. *A.* Closed and distended: marginal tentacle-buds just appearing on the whole circumference; slightly less advanced than the most oral portion of the two experimental pieces with tentacles. *B.* Closed and more or less distended but no tentacle-buds visible.

September 19. — 7 days after section. Experimental pieces: All closed and distended: marginal tentacles on the most oral portion of the oblique surface 2–3 mm.; from this region decreasing in length on each side, the lower three fourths to two thirds of the oblique surface still without tentacles.

Controls. *A.* Marginal tentacles 2–3 mm. about whole circumference. *B.* Marginal tentacles 1–1.5 mm. about whole circumference.

September 22. — 10 days after section. Experimental pieces: Marginal tentacles longest on upper side of oblique disc, decreasing to 0 toward lower side. In none of the three specimens are tentacles present on the lowest portion of the oblique disc: in one specimen the longest tentacles are 5–6 mm. and the circle of tentacles is nearly complete (Fig. 5); in the second the longest tentacles are 3–4 mm. and the lower one third of the disc is without tentacles; in the third the longest tentacles are 2–3 mm. and about one half of the disc is still without tentacles. This piece has been somewhat retarded throughout by irregular in-rolling at the aboral end delaying closure and distension.

On the three more advanced pieces labial tentacles are appearing on the upper one third to one half of the disc (Fig. 5).

Controls. *A.* Marginal tentacles 5–6 mm. about whole circumference. *B.* Marginal tentacles 3–4 about whole circumference.

September 26. — 14 days after section. Experimental pieces: In the most advanced piece (Fig. 6) the longest marginal tentacles on the upper side of the disc are 10 mm., the shortest on the lower side 2 mm. Labial tentacles are present on the upper two thirds of the disc, the longest being 3–4 mm. (Fig. 6).

In the second piece the tentacles are equal in length to those of the first and show a similar arrangement.

In the third (delayed) piece the longest marginal tentacles on the upper side of the disc are 8–9 mm. while on the extreme lower portion only minute buds are present. Labial tentacles are present on the upper half of the disc, the longest being 2–3 mm

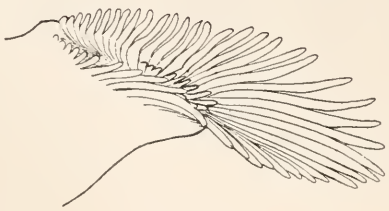


Fig. 5.

In all cases the disc is becoming less oblique (cf. Figs. 5 and 6).

Controls. *A*. Marginal tentacles 8–10 mm. about whole circumference. Labial tentacles somewhat irregular in length 1–4 mm. *B*. Marginal tentacles in one piece 8–9 mm., in other 5–6 mm. Labial tentacles in one piece 1–4 mm., in other about 1 mm.



Fig. 6.

Beyond this point the controls need not concern us. The experimental pieces were observed at intervals during three weeks more. During that time there was little increase in length of the longest tentacles, but the shorter tentacles continued to grow until the asymmetry was only slight: in other words the tentacles on the upper side of the disc completed their regeneration earlier than the others. Meanwhile the disc gradually became less and less oblique until it too was almost symmetrical in position. In all pieces the obliquity of the disc was much less

when the animal was distended and oriented in the characteristic position with oral end uppermost than when it was strongly contracted. In the later stages the disc did not appear at all oblique except in the contracted condition.

The rates of regeneration in the three experimental pieces were slightly different owing to the delay in closing in some, but if we take the most advanced of these pieces and compare it with the two controls it is seen that there is a marked difference between the lower portion of the disc in the experimental piece and the controls *B*. In the controls *B* the tentacles were 1–1.5 mm. seven days after section and must therefore have appeared on the sixth day, since they were not distinct on the fifth day. In the most advanced experimental piece the tentacles had not appeared on the lowest portion of the disc on the tenth day after section; four days later, however, they were about 2 mm. in length and must therefore have appeared about the twelfth day. There is then a delay of about six days in the appearance of the marginal tentacles on the lowest part of the oblique disk as compared with the time of appearance of the tentacles on a transverse disc at the same level of the body, and in the other experimental pieces the delay is still greater. It is evident then that the retardation in tentacle regeneration on the lower portion of an oblique piece is much greater than the retardation due to differences of level in transversely cut pieces. The data of the experiment and Figs. 5 and 6 show that the appearance of the labial tentacles is similarly delayed.

As regards the time of appearance of the marginal tentacles on the uppermost portion of the oblique disc and on the control pieces *A*, the fact that tentacles appeared in the most advanced experimental piece in three days, while in the controls they appeared somewhat later, indicates that tentacle regeneration is probably slightly accelerated in the upper portion of the oblique discs. According to my notes this is the only case observed in all of my experiments in which tentacles appeared in three days. It is barely possible that the oblique cut in this piece passed so far orally that small stumps of a few tentacles remained. In view of this possibility little stress can be laid on the apparent acceleration in tentacle-regeneration in this one piece, and unfortunately

I have at present no further evidence upon this point, as my attention was not directed to it early enough. I am inclined to believe, however, that there may be a slight acceleration here, provided the inrolling of the margins is not so irregular that closure is delayed, as is frequently the case in oblique pieces.

Leaving this point for future experiments to decide, the retardation of regeneration on the lower portions of the oblique discs is sufficiently evident and requires explanation. In the course of my experiments several other pieces were cut obliquely and always with the same result, viz., appearance of the tentacles first on the uppermost portion of the disc and from this region on each side in succession toward the lowest portion. The tentacles on the lowest portion always appeared later than in transversely cut pieces at the same level.

As in the case of typical regeneration from a transverse cut surface, the presence or absence of a cut aboral end and the plane of the cut if present do not affect the oral regeneration except in so far as irregular or delayed closure of the aboral end may delay distension.

It is difficult to conceive any reason for this retardation of regeneration in the organic relations of parts of the oblique piece. Why should there be any difference between a portion of an oblique disc and a transverse disc at the same level of the body? Yet tentacle-regeneration on the former requires about twice as much time as on the latter.

The only plausible reason for this difference that has occurred to me thus far is the difference in the relation between the inrolled margin and the circulatory currents in the oblique and transverse pieces. It is evident, I think, that as soon as the oblique piece becomes closed by new tissue, and distension and stretching of the body-wall occurs, the chief tension upon the inrolled margins will coincide in direction with the plane of the disc. Thus on the lowest portion of the oblique disc the direction of tension will be obliquely upward and on the highest portion it will be obliquely downward. The result is that at the lowest portion of the margin the disc forms an obtuse angle with the body-wall, while on the opposite side the angle is acute; between these points the angle varies between these limits. Examination of the specimens

shows this condition very clearly. At the uppermost portion of the disc the body-wall bends over sharply, almost as if actually folded, while the lower margin of the disc is rounded (Figs. 3 and 4) and the angle is much less.

It is evident that circulatory currents passing orally along the inner surface of the body-wall will exert much less pressure on the lower side than on the upper side, since the obtuse angle between disc and body-wall at the lower margin deflects them and permits escape toward the central portions of the body, while at the upper portion of the margin the currents strike an acute angle. On intermediate portions of the disc the angle, and consequently the pressure exerted, vary with the position from the maximum at the upper margins to the minimum at the lower margin. It is clear, moreover, that the angle between body-wall and disc at the lowest part of the margin of the oblique disc is greater than that between the body-wall and a transverse disc, while at the upper margin it is less. Half way between the upper and lower oblique margins it is the same as in the transverse disc.

If there is any relation between the circulatory currents and tentacle-regeneration we might expect acceleration of regeneration at the upper margin and retardation at the lower margin of the oblique disc. The retardation of regeneration is very evident, but as regards acceleration the data are insufficient to permit definite conclusions, though there are indications of a slight acceleration. In any case we could not expect the acceleration to be as great as the retardation since in ordinary cases of regeneration the inrolled margin forms a more or less acute angle with the body-wall and a difference in the size of this angle would make little difference in the pressure so long as the angle remains acute. As soon as the angle becomes obtuse however, the pressure must be reduced and as the angle increases the pressure becomes less.

In this, as in other cases, the regeneration of the labial tentacles proceeds in the same manner and sequence as that of the marginal tentacles, though much more slowly. As has already been mentioned, however, I do not know whether there is any localization of pressure in the regions where these tentacles appear.

My suggestions regarding the possible rôle of local pressure in bringing about the characteristic result in oblique pieces should be considered merely as an attempt to indicate how differences of local pressure may be effective here. The hypothesis must be applied to as many cases as possible before definite conclusions are reached. For the labial tentacles my data concerning local pressure are incomplete, as was pointed out in a previous paper ('04*b*). At present, however, I do not see any other probable explanation of the delay in the regeneration of the marginal tentacles on the lower portion of an oblique disc than that suggested above.

The gradual equalization in the length of the tentacles and the gradual reduction in the obliquity of the disc which are always features of the later history of oblique pieces, lead toward the establishment of symmetry and the typical form. Is there any satisfactory interpretation of these changes or must they be regarded for the present as inexplicable? They resemble in character certain of the phenomena which have led Driesch to assume the existence of an autonomistic principle or entelechy governing form. But I am convinced that an interpretation of an entirely different sort is possible.

In the first place it is necessary to recognize that the equalization of the tentacles and the reduction in obliquity of the disc are two wholly distinct phenomena due to wholly different factors. The equalization in length of the tentacles is the result of the same factors which cause the regeneration of tentacles of a certain length in typical transverse pieces and which bring about approximate equality in the length of tentacles in normal animals. If internal pressure plays a rôle in the latter cases, undoubtedly it is equally important in the former. Considering the oblique pieces, it is clear that as soon as the tentacles on the lowest part of the margin begin to grow conditions for their further growth are similar, as regards internal pressure and circulatory currents, to the conditions on the opposite margin. Each tentacle-bud forms a blind sac whose walls are growing tissue. Conditions of internal pressure in this sac do not differ on different parts of the margin, whatever the angle between disc and body-axis. The appearance of the tentacle-buds is delayed upon the lowest part

of the margins and the possible cause for this delay has been indicated above.

If the length of the tentacles is dependent upon internal pressure, either general or local, there is no reason why the tentacles on the lowest part of the margin should not finally attain the same length as those on the highest part. They will attain this length later than the others because the first stages in their regeneration are delayed. Other factors such as the difference due to difference in level (see Child, '03*b*) may prevent the complete equalization in length, but there is always a close approximation to equality. Evidently then this regulative process does not differ essentially from others which have been discussed. The same principles which were applied to those are applicable here. In fine, the equalization in length of the tentacles is exactly what might be expected if internal pressure is the chief factor in determining their length.

The equalization of the tentacles presupposes nearly equal growth in the tentacular region on all parts of the circumference but the reduction in obliquity of the disc is apparently a compensatory process. Either the body-wall beneath the lower portion of the disc must undergo increase in length, or the body-wall beneath the highest portion must undergo decrease in length, or finally, both these changes must occur in order that the disc may attain a position at right angles to the longitudinal axis. No consideration of the conditions of internal pressure will, in my opinion, afford any means of explaining this compensatory regulative change. On the other hand I think there is little doubt that it is the result of certain characteristic activities of the animal, viz., its orientation in space. Loeb ('91) called attention to the remarkable power of orientation in *Cerianthus* and showed that in whatever position it was placed the effort was made to bring the longitudinal axis or at least its oral portion into a vertical position, and that having once attained this position the animal remains quiet until other stimuli bring about movement. My own observations agree fully with Loeb's as regards this point. The ability of pieces lying on the flat bottom of aquaria to bend the body at right angles and so lift the oral portion into a vertical position is most striking and has formed a

constant feature of my experiments. In pieces with oblique discs an interesting modification of this orienting reaction has been observed. The oral portion of the body was brought into a vertical position and furthermore the effort was made by means of unequal contraction of the muscles on the two sides of the body to bring the disc into a horizontal position at right angles to the longitudinal axis. During the earliest stages of regeneration the pieces never react normally but after they become closed and distended the reactions gradually appear. In the oblique pieces this reaction is already very distinct at the stage of Fig. 5. When the pieces are left undisturbed they orient themselves so that the disc appears nearly horizontal and the body below it nearly vertical. That the muscles of the two sides must be unequally contracted is evident. A sudden tactile stimulus or other irritation causes a marked change in the relation of parts. The specimens undergo sudden contraction and the obliquity of the disc increases greatly. The stronger the stimulus and consequently the more complete the contraction of the muscles the greater the obliquity of the disc in such cases. Left to themselves, the animals gradually extend, become filled with water again and resume the former position. The increase of the obliquity of the disc in the contracted condition results from the difference in the degree of contraction at different points of the circumference in the distended oriented condition. A brief consideration will be sufficient to render this clear; in the normal animal a stimulus of this kind causes an equal degree of contraction in all the longitudinal muscles and there is no reason to suppose that the effect is different in the oblique pieces. The increase in the obliquity of the disc which accompanies contraction in oblique pieces must result from a difference in condition of the muscles on the opposite sides of the body. If for instance we suppose that the muscles of the body beneath the highest part of the oblique disc are partially contracted in the effort to bring the disc into a horizontal position it is evident that these muscles will contract less in consequence of the sudden stimulus than those on the opposite side of the body and consequently the angle between disc and longitudinal axis must decrease.

During the earlier stages of regulation the animal is unable to

bring the disc into a completely horizontal position : it always remains somewhat oblique. Contraction at this stage causes the angle between disc and axis to approach its original value, *i. e.*, the disc becomes nearly as oblique as the original cut surface. As time goes on, however, the oriented animal brings the disc more and more nearly into the horizontal position and the obliquity of the disc in the contracted condition gradually decreases. In the later stages the obliquity is visible only in the contracted condition, the disc being apparently at right angles to the longitudinal axis in the distended oriented specimen.

This relation between the distended, oriented condition and the contracted condition is characteristic not only for pieces with oblique discs but for various other forms artificially produced. Whenever the artificial form interferes with the orientation a gradual compensatory regulation may be observed.

The most obvious conclusion and, I think, the correct one, regarding the regulation of the oblique discs is that the changes in the body-wall of the two sides of the body occur in consequence of the characteristic functional condition of the muscles. The muscles beneath the upper side of the disc being continually more or less contracted undergo a "functional" change in structure and lose a part of their original power of extension, while the muscles beneath the lower edge of the disc, being continually highly extended lose to some extent their original power of contraction. Or, considered from another point of view, there is functional atrophy on one side and functional hypertrophy on the other. The ectoderm and the entoderm of the body-wall follow these changes by similar growth or reduction. With each change further change becomes possible until the animal is finally able to orient itself in the typical manner, or in other words has attained the typical form. The most important point in this interpretation is the rôle played by the efforts of the animal to carry on its typical activities. In my opinion these efforts are the cause of the regulation. The animal attempts to perform certain acts and certain resulting conditions of the tissues give rise to a certain form. The reaction or attempt at reaction produces the form incidentally. The typical form is then in this sense the result, not the cause of the typical reaction.

No attempt at general application of this conclusion to morphology need be made at present, though the field for speculation is most attractive. I desire merely to call attention to the fact that this regulative process belongs in the same category with the changes in form of pieces of *Stenostoma* in consequence of the attempts of these pieces to carry on their typical movements (Child, '02, '03). In both cases the form is, speaking broadly, the result, not the cause of the behavior.

The Regeneration of Pieces from the Œsophageal Region.

Pieces with the oral cut surface at some level of the œsophageal region of the parent specimen and the aboral end at a greater or less distance below the œsophagus differ from pieces cut wholly aboral to the œsophagus only as regards rapidity of regeneration. In such pieces, containing a part of the œsophagus, the cut oral end of this organ unites with the cut body-wall and thus a functional mouth is formed within a day or two after section. Consequently such pieces become fully distended earlier and the tentacles appear somewhat sooner than in pieces in which a new mouth is regenerated.

In pieces representing only the œsophageal region or part of it (Fig. 7) the result usually differs widely from the preceding. In such pieces the cut ends of the œsophagus usually unite both orally and aborally with the cut body-wall and the œsophagus forms a tube extending completely through the piece and open at both ends to the exterior. Fig. 8 represents a longitudinal section of such a piece : in order that the œsophagus and body-wall may be readily distinguished in the diagrams the thin muscular layer of the former is not indicated. In these pieces there is no connection between the œsophagus and the enteron ; water passing into the oral end of the œsophagus simply passes out again at its aboral end. Each intermesenterial chamber is thus shut off from all communication with the exterior and with adjacent chambers.

As regards tentacle-regeneration these pieces follow the usual course during the first few days, becoming more or less distended with fluid, which is secreted into the enteric cavity or diffuses

through the body-wall. The body-wall becomes thinner at the oral end (Fig. 9) and tentacle-buds appear (Fig. 10), but these usually do not develop beyond the stage shown in Fig. 10. Occasionally they reach a length of 2–3 mm., but further than this their development never proceeds. The distension gradually decreases again after a week or two and the tentacles which



FIG. 8.



FIG. 9.

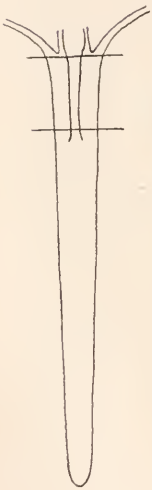


FIG. 7.



FIG. 10.



FIG. 11.



FIG. 12.

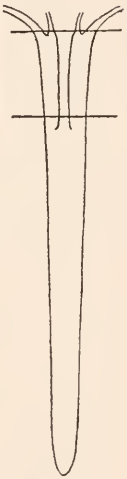


FIG. 15.



FIG. 13.



FIG. 14.

had attained a length of 2–3 mm. decrease in size to mere buds. In cases where the tentacles never develop beyond the stage represented in Fig. 10 they are scarcely visible at all after two or three weeks.

In some pieces, however, closure of the aboral end occurs sooner or later. This result may be attained in either of two ways. In one case the aboral cut surfaces of the œsophagus and body-wall fail to come into contact (Fig. 11) and the aboral end closes in the usual manner by union of the cut surfaces of the body-

wall leaving the aboral end of the œsophagus free in the enteron (Fig. 14). Such pieces do not differ essentially from pieces in which the aboral end lies below the œsophageal region. They become distended in the usual manner and regenerate typically, but more rapidly than pieces in which regeneration of mouth and œsophagus occurs.

In the other case the cut surfaces of body-wall and œsophagus unite in the manner described above (Fig. 8), either completely or on a part of the circumference. Then it may happen that different portions of this region of growing tissue are brought into contact as in Fig. 12. The result is the union of all these parts and so the closure of the aboral end (Fig. 13). The connection between the œsophagus and body-wall is soon broken as the piece becomes more fully distended and the condition represented in Fig. 14 is attained. From this stage on regeneration at both ends proceeds in the typical manner. In some cases this closure of the aboral end and loss of connection between œsophagus and body-wall does not occur at first, but later the margins of the body-wall happen to come into contact perhaps in consequence of the gradual decrease in distension mentioned above as following the first increase, and thus closure occurs and is followed by renewed distension and rapid regeneration. Apparently the new tissue retains the power of making new unions for a considerable time after the cut surfaces of body-wall and œsophagus appear to be firmly united.

The final result in all œsophageal pieces in which the aboral end succeeds in closing aboral to the œsophagus is typical regulation with well-developed tentacles at the oral end and several millimeters of new tissue at the aboral end.

The data of a few experiments will serve to indicate the uniformity of results. In all cases *C. solitarius* was used.

Series 30.

September 28, 1902. — Seven œsophageal pieces were prepared from large specimens in good condition as follows: at the oral end of each animal a transverse cut was made through the disc at such a level that the marginal tentacles and margins of the disc were removed and the labial tentacles were cut off near their

bases leaving stumps 1 mm. or less in length. Then a second cut was made through the œsophageal region near its aboral end. In the distal pieces thus formed the œsophagus extends from end to end of the piece, cut surfaces being present at each end (Fig. 15).

October 1. — 3 days after section. In all cases the cut margins of œsophagus and body-wall have united both orally and aborally in the same manner as in Fig. 8. All pieces are more or less distended and the oral margins slightly crenated in correspondence with the intermesenterial chambers.

October 4. — 6 days after section. All pieces with marginal tentacles, varying in length in different pieces from mere buds just visible to 2 mm.: labial tentacles still short stumps.

October 7. — 9 days after section. In two pieces the aboral end has closed as in Figures 12–14: these pieces fully distended; one with marginal tentacles 5 mm.; labial tentacles about 2 mm.; the other with marginal tentacles 3 mm., labial tentacles 1–1.5 mm.

In the five remaining pieces no aboral closure has occurred; the distension has decreased; the marginal tentacles have increased in length, though very slightly, since the previous examination, varying from minute buds to 1–2 mm.

October 15. — 17 days after section. One of the pieces which closed aborally with marginal tentacles 8–10 mm., labial tentacles 5–6 mm. At aboral end new tissue 1–2 mm.

The other closed piece was lost.

In none of the five remaining pieces has tentacle-regeneration proceeded since the previous examination. All appear almost completely collapsed and the tentacles are reduced to mere buds in all, being scarcely visible in some. One piece has begun to break up.

During the following two weeks the five pieces which did not close broke up into small pieces and died, a frequent occurrence in small pieces in which closure and regeneration do not occur. The one closed piece remained in good condition.

The difference between the piece that closed and the other five is striking. None of these five pieces ever regenerated marginal tentacles more than 1–2 mm. in length, and practically no regeneration of labial tentacles occurred. Yet the one piece which

closed while no longer than these and differing from them only in that the œsophagus communicated with the enteron, regenerated marginal tentacles 8–10 mm. in length and labial tentacles 5–6 mm. and produced new tissue at the aboral end.

Series 32.

October 1, 1902. — Seventeen œsophageal pieces were prepared (Fig. 7) the distal cut being in this case slightly more aboral than in Series 30 (Fig. 15) so that both marginal and labial tentacles and most of the surface of the disc were removed.

During two weeks these were examined several times. In all pieces the cut margins of œsophagus and body-wall united both orally and aborally and in none did the closure of the body-wall across the aboral end occur. All of the pieces became slightly distended during the first few days and marginal tentacles appeared as minute buds less than 1 mm. in length. In no case did regeneration proceed beyond this stage: labial tentacles never appeared. At the end of two weeks some pieces were beginning to break up.

Series 47.

November 7, 1902. — Nine œsophageal pieces were prepared in the same manner as those of Series 32.

November 10. — 3 days after section. Still more or less completely collapsed: no tentacles visible on any.

November 12. — 5 days after section. One piece closed aborally, and well-filled with water; marginal tentacle-buds 0.5–1 mm. In the remaining eight pieces œsophagus and body-wall have apparently united both orally and aborally: these pieces are only slightly distended and show a slight crenation of the oral margin corresponding to the intermesenterial chambers.

November 20. — 13 days after section. During the interval since the last examination seven pieces closed aborally across the end of the œsophagus. These are fully distended and bear marginal tentacles 2–3 mm.; labial tentacles just appearing.

In two pieces the œsophagus remained open aborally. These are not fully distended and the marginal tentacles are minute buds about 0.5 mm.; labial tentacles are absent.

December 2. — 23 days after section. In the seven closed

pieces regeneration has proceeded in the typical manner; marginal tentacles 5–6 mm.; labial tentacles 2–3 mm.

In the other two pieces regeneration has proceeded no further. They are still only slightly distended and with minute tentacle-buds.

In this series a larger proportion of the pieces closed aborally across the end of the œsophagus than in any other series of this kind. The date of this series was later in the year than that of any other, *i. e.*, the temperature of the water was lower and the distension of the pieces occurred much more slowly (see Child, '03*b*). It is probable that different parts of the aboral cut surfaces of the body-wall remained in contact for a longer time than in the other cases where distension occurred more rapidly, and that this prolonged contact made union possible in a greater number of cases than in other series. Other conditions such as difference in the degree of contraction of body-wall and œsophagus at the time the lower cut was made may have aided in bringing about this difference. However that may be, the point of chief importance, viz., the difference in regeneration between those pieces which did close aborally across the œsophagus and those which did not is as clear in this series as in others.

While the general result of these experiments is sufficiently clear special attention may be called to certain points.

In all cases in which the œsophagus remains open aborally and consequently does not communicate with the enteron the pieces never become fully distended and regeneration is slight. Pieces cut below the œsophageal region become well distended and the marginal tentacles may attain a length of 2–3 mm. before the mouth is formed. In my first paper ('03*a*) diffusion of water through the body-wall in consequence of the presence of soluble products of metabolism or other substances in the enteron was suggested as a possible cause of this first distension. It is also possible that secretion of fluid into the enteron occurs. In the œsophageal region the entodermal layer is thinner and undoubtedly of less functional importance than in the region aboral to the œsophagus, and it is reasonable to suppose that the accumulation of fluid in the œsophageal pieces would be much less rapid than in pieces from regions aboral to it. If this be

admitted it follows that less water would enter these pieces and less distension would occur than in pieces aboral to the œsophagus. If the internal pressure affects tentacle-regeneration, less regeneration would occur in consequence of this slight distension in œsophageal pieces than in others where distension is greater, and this is actually the case.

It was noted that the pieces in which the œsophagus remains open aborally gradually collapse again in the course of a week or two. This collapse is probably due to a slow loss of the fluid which first caused the distension. If the body-wall is slightly permeable for substances in solution in the enteron the distension produced at first will gradually diminish since in consequence of continued starvation the amount produced gradually decreases. Whatever the exact nature of the process may be, a decrease in the distension occurs. As this process continues the regenerating tentacles also decrease in size instead of continuing to grow. This fact is important as showing the apparent close relation between tentacle-regeneration and internal water-pressure. In pieces cut below the œsophagus the regeneration of the mouth and the entrance of water through it serves not only to prevent decrease in the internal pressure but to increase it, and further growth of the tentacles takes place.

The most striking feature of the experiments is the difference in behavior as regards regeneration between the pieces in which the body-wall closes aborally across the œsophagus and those in which the œsophagus remains open aborally to the exterior. The pieces of the first kind begin to regenerate typically as soon as the closure occurs, while the others never produce anything more than marginal tentacles 1–2 mm. in length. In the introductory description of these experiments it was shown that in case of closure the connection between the aboral end of the body-wall and the œsophagus is severed and the œsophagus opens into the enteron (Figs. 12–14). The breaking of this connection, *i. e.*, the change from the condition shown in Fig. 13 to that of Fig. 14 is probably itself due at least in part to internal water-pressure, though the manner of its occurrence cannot be observed. Other factors, such as a difference in rapidity of growth or a difference in resistance between the tissue of the

œsophagus and that of the body-wall may also play a part in the result. Whatever be the cause, the result is free communication between a fully formed œsophagus and the small enteric cavity with its intermesenterial divisions. If entrance of water through the œsophagus serves to maintain internal pressure in *Cerianthus* it is clear that in these pieces the pressure should at once become about equal to the pressure in a normal animal since the mouth opening and œsophagus are not regenerated structures but parts of the parent body and of full size. If internal water pressure affects regeneration we might expect in these pieces rapid regeneration, even more rapid than in pieces with regenerating mouth and œsophagus. Close comparison of these pieces with others in which mouth and œsophagus are formed by regeneration is difficult, since in the œsophageal pieces regeneration may be at first delayed until the aboral closure is complete, but the piece in Series 30 in which closure of the aboral end occurred between October 4 and October 7 and by October 15 the marginal tentacles were 8–10 mm. and the labial tentacles 5–6 mm., show the rapidity of regeneration. In fact regeneration in this piece after closure is more rapid than in any other case noted in my records with the exception of other pieces of the same kind.

The striking difference between œsophageal pieces in which the aboral closure occurs and those in which it does not constitutes evidence of great importance for the influence of internal water-pressure on regeneration. As regards the influence of local pressure due to circulatory currents in determining the position of tentacles and in their regeneration these experiments afford no direct evidence. They show merely that the greater the distension of the piece and consequently the more widely open the intermesenterial chambers and the greater the force and volume of the circulatory currents the more rapid is regeneration.

Summary.

1. In pieces with oblique oral end the rapidity of tentacle regeneration differs on different parts of the disc, being greatest on the uppermost (most oral) portion and least on the lowest (most aboral) portion. The delay in regeneration on the lowest portion

as compared with the highest portion is much greater than the difference in rapidity of regeneration due to difference in level.

2. The only reason apparent for the difference in rapidity of regeneration on different parts of oblique discs is the difference in the angle between disc and body-wall, which is acute on the upper side of the disc while on the lower side it is obtuse. In consequence of this difference the local pressure exerted by the circulatory currents passing orally in the intermesenterial chambers must be much greater on the upper side of the disc than on the lower. If regeneration is influenced by these currents we should expect delay on the lower side, and it occurs in all cases.

3. The later equalization in length of the tentacles in pieces with oblique discs is due to the conditions which bring about equality in length of tentacles in normal animals. After the appearance of the tentacles the conditions as regards internal pressure are essentially similar on all parts of the oblique margin and equalization must be expected if the length of the tentacles is determined by internal pressure.

4. The reduction in the obliquity of the disc in oblique pieces is a compensatory process resulting from the attempt of the animal to orient itself with longitudinal axis vertical and disc horizontal. In the attempt at orientation unequal contraction of the muscles on different parts of the circumference occurs, and its continuation brings about changes in the tissues which lead gradually toward the establishment of the typical form. The form is the result, not the cause of the reaction.

5. In pieces cut wholly within the œsophageal region the two cut ends of the œsophagus usually unite with the cut ends of the body-wall, thus leaving the œsophagus open to the exterior at both ends. This method of closure isolates each intermesenterial chamber completely and prevents any direct communication between the enteron and the exterior.

6. Such œsophageal pieces become slightly distended at first in consequence of diffusion of water through the walls, but since the œsophagus is not in communication with the enteron the internal pressure remains far below that of the normal animal. Regeneration of the marginal tentacles begins in such pieces, but never proceeds beyond the formation of mere buds.

7. Occasionally an œsophageal piece closes aborally by union of the body-wall across the end of the œsophagus. In these cases communication between the œsophagus and enteron is established, the piece becomes fully distended, and regeneration proceeds in the typical manner.

8. The difference in regeneration between the "closed" and "open" œsophageal pieces is undoubtedly due to the difference in the degree of internal water-pressure.

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO, November, 1903.

BIBLIOGRAPHY.

Child, C. M.

'02 Studies on Regulation, I. Fission and Regulation in Stenostoma. Arch. f. Entwickelungsmech., Bd. XV., H. 2, 1902.

'03 Studies on Regulation, II. Experimental Control of Form-Regulation in Zoöids and Pieces of Stenostoma. Arch. f. Entwickelungsmech., Bd. XV., H. 4, 1903.

'03a Form-Regulation in Cerianthus, I. The Typical Course of Regeneration. Biol. Bull., Vol. V., No. 5, 1903.

'03b Form-Regulation in Cerianthus, II. The Effect of Form, Position, Size, and Other Factors upon Regeneration. Biol. Bull., Vol. V., No. 6, Vol. VI., No. 1, 1903.

'04a Form-Regulation in Cerianthus, III. The Initiation of Regeneration. Biol. Bull., Vol. VI., No. 2, 1904.

'04b Form Regulation in Cerianthus, IV. The Rôle of Water-Pressure in Regeneration. Biol. Bull., Vol. VI., No. 6, 1904.

'04c Form-Regulation in Cerianthus, V. The Rôle of Water-Pressure in Regeneration : Further Experiments. Biol. Bull., Vol. VII., No. 3, 1904.

PORTABLE ANT-NESTS.

ADELE M. FIELDE.

Portable ant-nests, constructed by me in the summer of 1900, were described in Biological Bulletin, No. 2 of Vol. II., which is now out of print. Improvements that have since been made in them, and their present use by myrmicologists in America, Europe and Africa, justify a new statement of the method of making them. I have now ants that have lived in them, without earth, for three years, in health and apparent contentment.

The floor of the nest is a pane of double-thick, transparent glass. This is laid upon very thick, white blotting paper, giving an elastic bed to the pane of glass and the best background for observation of the ants. The paper has just the area of the glass, but is not fastened thereto.

The outer walls of the nest are laid a quarter inch, or six millimeters, from the edge of the pane. They consist of two strips of double-thick glass, a half inch, or thirteen millimeters, wide, the one strip superimposed on the other. Both are held in place by crockery cement.[1] The wall is smoothly laid up, with no interstices where an ant may hide or escape.

The partitions are double the width of the wall, which they otherwise copy. At one end of every partition a space is left whereby the ants may pass from room to room. This passage-way is covered by a thin celluloid film or a piece of mica. It is desirable that this covering be transparent, so that the passage-way underneath it may be scanned from above, on lifting the end of the toweling which is to overlay it.

After the cement is well dried, the edge of the floor-pane and the outside of the walls are covered with a fabric impervious to light. Cloth serves better for this purpose than does paper, the edges of the nest being subject to much handling. Le Page's or

[1] The use of cement instead of glue was recommended by Dr. W. M. Wheeler. Diamond, Major's or any reliable kind may be used. I have merged in water, for two weeks, a nest constructed with Major's cement, without loosening its parts. The directions accompanying the selected cement should be followed

some other good liquid glue is used for securing the fabric upon the walls.

The walls and partitions are topped by Turkish toweling of a sleazy sort, folded over one layer of cotton wadding so that the edges of the strip of toweling meet in the center of the under side of the wadding. The wadding is cut to the same width as the wall or the partition. The toweling is just twice the width of the wadding, and its edges are basted evenly together, making a cushion of even thickness. It serves the double purpose of admitting air into the nest and of preventing the escape of the ants between the roof and its supports. It is held taut and is made level; is fitted snugly at the corners; exhibits no ravelings to afflict the ants; and is firmly glued to the glass beneath it. When a cushion becomes soiled by long use of the nest, the glue may be softened by soaking and the cushion may be removed and be replaced by a new one. The ends of the cushions are fringed out a half inch or more, and are left open so that the enclosed wadding may be adjusted to present a perfectly level surface.

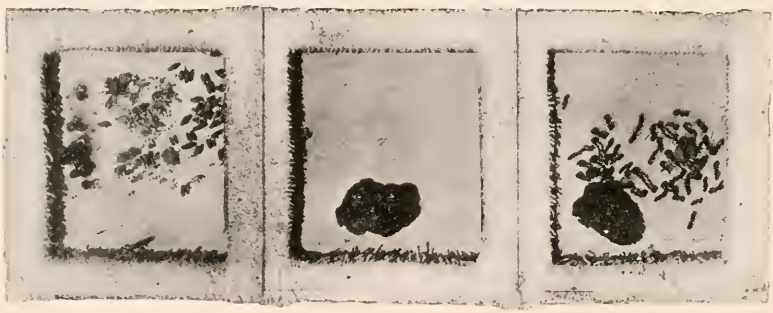
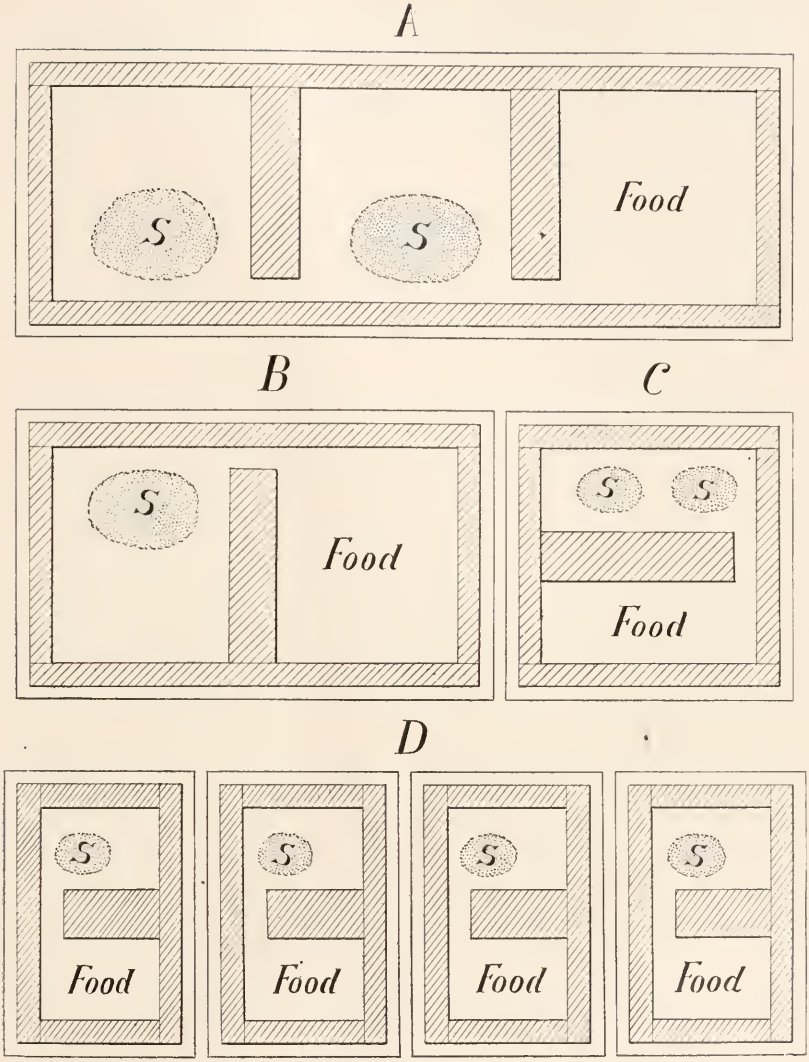


FIG. 2. The A nest completed.

There is a glass roof-pane for each room in the nest. The glass is thin; extends to the middle of the partition and to the outer edges of the walls on which it rests; prevents the exit of ants; and permits observation of their behavior. The glass may be without color, or it may be of a red or orange tint that will partially exclude ultra-violet rays of light. Ants perceive only such rays of light as are of short wave-length and, by use of a spectroscope, a glass roofing may be selected which renders the



Bases of Seven Ant-nests, Filling Three Shelves of Portable Case.

Fig. 1.

ants visible within the nest while it protects them from such light-rays as they instinctively shun.[1] If such glass is used for roofing the nest, the ants will behave as if in the darkness where they habitually live.

An outer roofing of blotting-paper makes the interior of the nest wholly dark. The food-room should be light, as it represents the ant's outside world.

When any room in the nest requires cleaning, it is covered only with transparent glass, and then the ants withdraw from it with their young into a dark room, which may in its turn be made light.

The food-room is dry, and in cool weather requires attention but once a fortnight. Sponge-cake merged in a little honey or molasses, banana, apple, mashed walnut, and the muscular parts and larvæ of insects are among their favorite edibles. Food is constantly attainable in the nest, but it is introduced in tiny morsels that it may not vitiate the air.

Since moisture encourages the growth of mold, no water is put into the food-room. But ants often drink, and they require a humid atmosphere. All other rooms than that allotted to their food are made humid by laying a flake of sponge on the floor and keeping the sponge saturated with clean water dropped twice a week from a pipette. The proportion of the floor which is covered by the sponge depends on the degree of moisture in the soil usually chosen as the habitat of the species. The sponges are kept clean by weekly washing and an occasional immersion in alcohol. Sponges of fine tough texture render best service, as they offer no apertures where the ants may conceal their eggs. The flake of sponge is so thin as to permit the ants to pass between it and the roof-pane.

The completed nest is less than half an inch or thirteen millimeters in its interior height, and does not exceed three fourths of an inch or two centimeters in its exterior height. A low-power lens is easily focused upon the ants within the nest.

During four years of experimental work with ants, I have found that the nests whose bases are represented in the drawing

[1] "Supplementary Notes on an Ant," Adele M. Fielde, *Proceedings of the Academy of Natural Sciences of Philadelphia*, June, 1903, p. 492.

meet the requirements of the various species that I have desired to house for long periods.

A is	16¼	× 6	inches or	41	× 15	centimeters.
B "	10	× 6	"	25½	× 15	"
C "	6	× 6	"	15	× 15	"
D "	6	× 4	"	15	× 10	"

Nests of these dimensions fit into and fill a portable wooden case or box having an interior length of 17 inches or 43 centimeters; a width of 7 inches or 18 centimeters; and a height of 4¾ inches or 12 centimeters. It is made of half-inch pine boards, dovetailed at the joinings. The interior of the case is equally divided lengthwise into four compartments by three shelves that are supported in grooves cut in the end-pieces of the case. The shelves are a quarter inch or six millimeters thick, and they leave space at their outer edge for the inset of a door which forms the front of the case, is hung by hinges at the bottom, and is held shut by two buttons affixed to the top-piece of the case.



FIG. 3. Portable Case for the Nests.

Holes are bored in both sides of the case for the inlet of air to the enclosed nests.

A handle placed lengthwise on the top of the case permits its convenient carriage. When its four compartments are filled with

nests, its weight is less than sixteen pounds. Ants have made long journeys in my portable nests, with no grave disturbance of their domestic arrangements.[1]

When preparing the nests for a journey, tapes are tied around them, so as to hold the roof-panes securely in position, and bits of wadding are so inserted as to prevent their displacement in the case. Four of the A nests, sixteen of the D nests, or a selection from among the A, B, C and D nests may be carried in the case.

Before constructing my ant-nests, I made and used those of the Lubbock and of the Janet patterns, both much older than my own. The Lubbock nest, holding the ants on an island by a moat filled with water, is not portable ; and whenever the pane of glass, covering the layer of earth over the island, needs to be cleaned, there must be a disturbance of the domestic interests of the ants. But the base of the Lubbock nest is a valuable adjunct when the ants are to be housed in my nests. It consists of a square or oblong block of wood, about two inches or five centimeters thick, with a channel grooved to half the thickness of the wood at a half inch from its edge all around. When the channel, which is an inch or more in width, is filled with water, the island thus formed serves well for the temporary confinement of ants. The ants are brought, as Janet suggested, from their wild nests in little bags permeable by air, or in jars whose mouths are covered by gauze. The contents of the bag or jar are deposited thinly upon the island ; a piece of glass covered by blotting paper and raised slightly above the general surface is laid over some portion of the area ; and the ants, within a few hours, gather the young underneath the darkened glass. Their progress in their work is made visible by an instant's lifting of the blotting paper. Selections from the total capture may be made for removal to the glass nests. My nests of earlier construction, like the Janet nests, had an aperture in the wall through which the ants could themselves transport their young from the earth into the glass nest. But I have found it expedient to personally make selection from the total capture rather than to allow the ants to bring their whole community into the glass nest.

[1] The photographs with which this paper is illustrated were very kindly made for me by Mr. J. G. Hubbard and Dr. O. S. Strong.

Forel's method of making, upon a table, a stockade of dry plaster of Paris, to prevent the escape of the ants deposited within it, serves well when large colonies are dealt with. But ants have high regard for personal cleanliness and are discomforted by the adhering dust which punishes their effort to escape over this stockade. The Lubbock island renders clean stock.

The Janet nest, a series of four pits in porous stone, cement or stucco, with water in the pit at the end opposite the food-pit, proved that the ants may live healthfully without earth; showed the practical value of more than one compartment in the artificial nest; and gave an excellent background for viewing the ants. But the Janet nest is cumbrous and weighty; and its food room becomes quickly mouldy in hot weather.

The glass nests are constructed at less expense than are those of either the Lubbock or the Janet pattern; they are easily kept clean, and the small space which they occupy, with their very light weight, greatly facilitates the bringing of the ants under close observation.

The Marine Biological Laboratory of Woods Holl, Mass.,
July, 1904.

EXPERIMENTS ON THE ORIGIN OF THE CLEAVAGE CENTROSOMES.

EDWIN G. CONKLIN.

(From the Zoölogical Laboratory of the University of Pennsylvania.)

The great diversity of opinion as to the origin of the cleavage centrosomes which appear during the fecundation of the egg is well known to all students of cytology. By different authors every possible view has been held with regard to the source of these centers, as may be seen from the following classification:

1. The cleavage centrosomes come from the sperm centrosome; Boveri ('87, '92, '95), Vejdovsky ('88), Fick ('93), Wilson and Mathews ('95), Hill ('95), Mead ('95), Reinke ('95), Kostanecki and Wierzejski ('96), Kostanecki and Siedlecki ('96), Sobotta ('97), MacFarland ('97), Erlanger ('97), Griffin ('99), Coe ('99), Linville (1900).

2. The cleavage centrosomes come from the egg centrosome; Van Beneden ('87), Wheeler ('95); all cases of normal and artificial parthenogenesis.

3. The cleavage centrosomes come from both egg and sperm centrosomes; Fol ('91), Guignard ('91), Blanc ('93), Conklin ('94), Carnoy and Lebrun ('99).

4. The cleavage centrosomes come from neither egg nor sperm centers but are new formations; Foot ('97), Lillie ('97), Child ('97), Mead ('98).[1]

It is probable that some of these conflicting views may be attributed to erroneous observations, and the writers who have maintained the first view have in general explained all others as due to this one cause, but on the other hand some of the evidence in favor of these other views cannot be thus lightly brushed aside. As long as this question remained on a purely observational basis no one seems to have seriously considered that there might be an element of truth in more than one of these views and that the cleavage centrosomes might arise differently in differ-

[1] For references see Wilson, "The Cell in Development and Inheritance," 1900.

ent animals or even in the same animal under different conditions.

The experiments of R. Hertwig, Morgan, Loeb and Wilson have shown that the unfertilized egg is capable of giving rise to cleavage centrosomes and spindles, while the observations of Delage, Boveri and others on merogeny have proven that a normal mitotic figure may appear in connection with the sperm nucleus in enucleated egg fragments. Under these circumstances it ought to be possible, by slightly altering normal conditions, to bring about the formation of a spindle in connection with each germ nucleus.

In the summer of 1901, while at the Marine Biological Laboratory at Woods Holl, I undertook some experiments on the eggs of *Crepidula* in order among other things to test this possibility. Since this animal is one which does not conform to the prevalent view as to the origin of the cleavage centrosomes it seemed all the more favorable for such work. As the eggs of these gasteropods are fertilized while still in the oviduct I have found it impracticable to experiment with unfertilized eggs but have worked entirely with eggs into which a spermatozoön had already entered.

The normal course of the fecundation in this animal may be briefly recalled: After both polar bodies have been extruded the egg nucleus lies at the animal pole in an area of cytoplasm while the sperm nucleus lies in the yolk near the periphery of the egg and usually near the vegetative pole. Then the egg centrosome which is left in the egg at the close of the second maturation division, rapidly disappears and in its place is left the large egg aster or sphere. At no stage is there a clearly marked sperm centrosome, but a radiating cytoplasmic figure, the sperm aster, develops in connection with the sperm nucleus and these two migrate through the yolk until they come into contact with the egg nucleus and sphere, immediately under the polar bodies. Here the egg and sperm spheres fuse and at their periphery two separate and independent centrosomes appear which ultimately come to lie at opposite poles of the first cleavage spindle. It is probable that one of these centrosomes comes from the egg sphere and the other from the sperm sphere though there is no

positive evidence that they are directly derived from egg and sperm centrosomes.

If now the eggs of *Crepidula plana* which have given off the second polar body but a short time before are brought for four hours into a 1 per cent. solution of NaCl in normal sea water

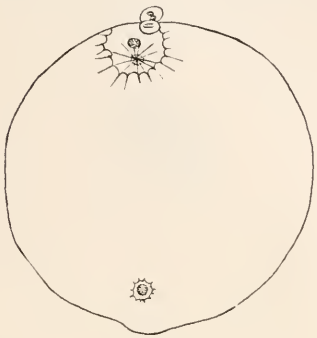


FIG. 1.

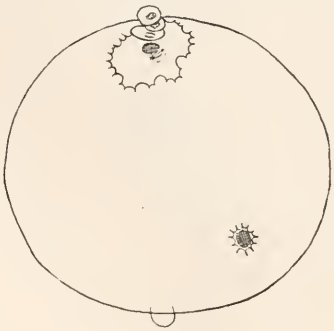


FIG. 2.

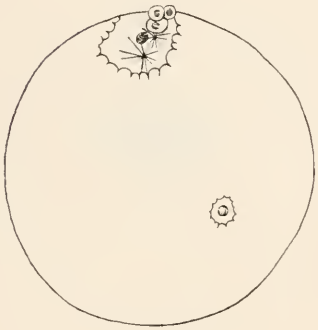


FIG. 3.

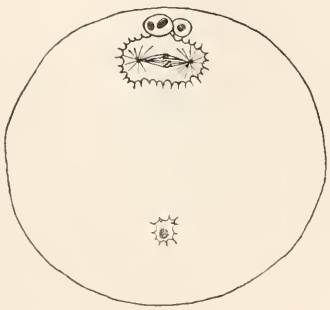


FIG. 4.

FIGS. 1–4. Eggs of *Crepidula plana* treated with 1 per cent. NaCl in sea water for four hours, viewed from the side. In all the eggs the polar bodies are at the upper pole, the egg nucleus and centrosome, or spindle, immediately below this, while the sperm nucleus lies in a small area of cytoplasm near the lower pole. Various stages in the formation of the egg spindle are shown.

they will present the appearances shown in Figs. 1–12. These figures are camera drawings of eggs in different stages of the formation of the first cleavage spindle and all are from the same experiment.

An examination of these figures shows that various stages in the division of the egg centrosome occur (Figs. 1–5) leading to

the formation of a perfect spindle of small size. There can be no doubt that the centrosomes of this spindle are derived from the egg centrosome. The sperm nucleus is at this time far removed from the egg nucleus and is closely surrounded by yolk; no astral radiations are found in connection with it, though one

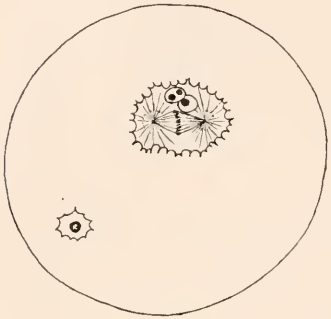


FIG. 5.

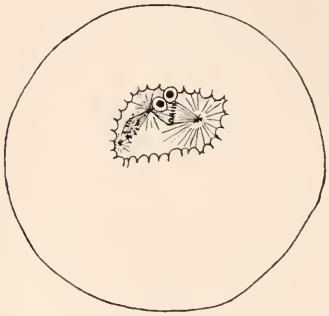


FIG. 6.

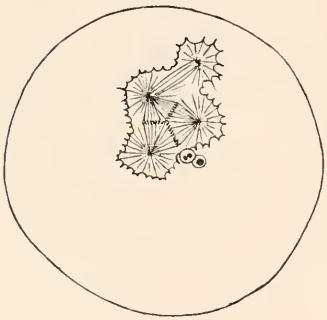


FIG. 7.

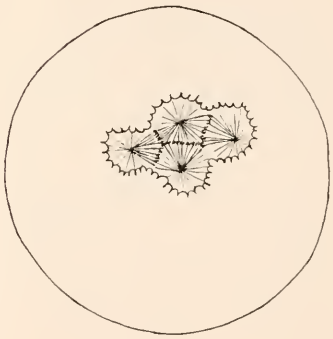


FIG. 8.

FIGS. 5–8. NaCl eggs of *C. plana* viewed from the animal pole; the two polar bodies are shown nearly over the spindles in the first three figures. In Fig. 5 the sperm nucleus is still some distance from the egg spindle; Fig. 6 shows an egg spindle and a sperm spindle joined at one pole; Figs. 7 and 8 show stages in the formation of a tetraster from the egg and sperm spindles.

or two granules which lie close to the nuclei may possibly represent centrosomes (Fig. 4).

Unfortunately a gap occurs at this stage in my material; in all the preceding figures (1–5) the sperm nucleus is small and densely chromatic and is far removed from the animal pole; in the succeeding figures (6–12) the sperm nucleus has been re-

solved into chromosomes which lie near those of the egg. In all cases there are two spindles present which are usually united into a tetraster though they may be more or less independent of each other. In some eggs yolk spherules separate the two spindles so that a tetraster is not formed (Figs. 9, 10), while in others an incomplete tetraster is formed (Figs. 6, 7); in still

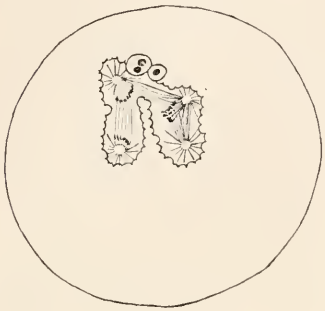


FIG. 9.

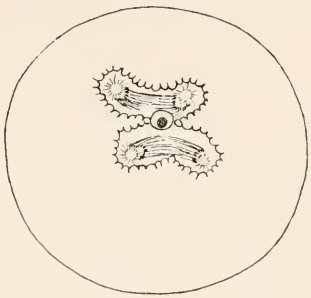


FIG. 10.

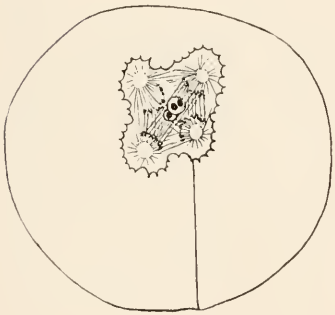


FIG. 11.

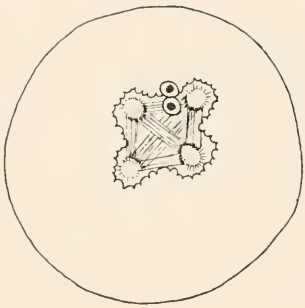


FIG. 12.

FIGS. 9–12. NaCl eggs of *C. plana*, viewed from the animal pole; the polar bodies lie above the spindles in all the figures. Fig. 9. Egg and sperm spindles united at one pole. Fig. 10. The two spindles quite separate. Figs. 11 and 12. Complete tetrasters in different phases of the separation of the chromosomes.

others the tetraster is complete, each of the four poles being united to the other three (Figs. 8, 11, 12).

Although I have not seen the genesis of the sperm spindle there seems to be no reason for doubting that it is formed essentially as the egg spindle is and that the two are at first wholly independent; only later do secondarily formed fibers connect one

or both of its poles with those of the egg spindle. Since under normal conditions one of the cleavage centrosomes of *Crepidula* arises in connection with the egg and the other with the sperm nucleus the only respect in which these experimental results differ from the normal is that in the former the centrosomes divide while the nuclei are still far apart thus giving rise to two spindles or to a tetraster, whereas in the latter these centrosomes do not appear until after the germ nuclei and spheres have met and they do not divide until the prophase of the second cleavage.

In such a case as this we have essentially the same phenomenon as is found in dispermic echinoderm eggs (Driesch, Boveri, Wilson) save that in the former the two additional centrosomes are not derived from an additional spermatozoön but come from the egg centrosome. Of this fact there cannot be a particle of doubt and it seems to me to shed light upon the much discussed question as to the source of the cleavage centrosomes of different animals under normal conditions.

It has evidently been a mistake to suppose that the cleavage centrosomes could arise in but a single way and that all animals must conform to this single type. Under experimental conditions the cleavage centrosomes may arise in one and the same animal in connection with the sperm nucleus, in connection with the egg nucleus or, as I have shown in connection with both of these nuclei, while it is possible that they may arise *de novo* anywhere in the egg cytoplasm, though this latter view is by no means so well supported by evidence as are the former ones (see Conklin 1902). It is highly probable therefore that the source of the cleavage centrosomes may differ in different animals, or even in the same animal under different conditions.

It is interesting to note that this whole discussion as to the supposed importance of the source of the cleavage centrosomes had its origin in the thought that the sex cells were in themselves incomplete and incapable of development save as each complemented the other (Boveri '87, '91), or in the rival notion that the centrosomes were the bearers of heritable qualities (Fol '91). In the light of recent experimental work both of the these views are seen to be untenable and the subject has therefore lost most of its interest and significance.

Vol. VII. *October, 1904.* *No. 5*

BIOLOGICAL BULLETIN

POWER OF RECOGNITION AMONG ANTS.

ADELE M. FIELDE.

With Photographic Illustrations by Mr. J. G. Hubbard and Dr. O. S. Strong.

When power of recognition is to be tested, appeal for evidence of that power is properly made through the leading sense. We should take evidence from the eagle through its sense of sight, from the mole through its sense of hearing, from the caterpillar through its sense of touch, from the ant through its sense of smell.

That ants have associative percepts which are independent of their chemical sense, is proven by their behavior. Ants learn to be unafraid of the light from which they instinctively withdraw their young.[1] When ants are put into an artificial nest, some weeks are required for their making acquaintance with their domicile, but after such acquaintance has been perfected, they may be transferred to a replica of their abode, whether it be a Petri cell, a glass house, or a wooden box, and they will be wholly at ease in it, and will quietly resume their accustomed routes over its floors or withdraw into it from a strange environment, although it lacks their nest-aura.

I divided a small colony of *Camponotus Pennsylvanicus* into two sections. I fed and fondled the members of the one section until they manifested a sense of safety in my presence, would mount my finger and make a leisurely promenade upon my hand or would return to me after an hour's wandering in my room. These ants not only ceased from biting me when I took them upon my hand, but became so tame that they would

[1] "Supplementary Notes on an Ant," A. M. Fielde, *Proceedings of the Academy of Natural Sciences of Philadelphia*, September, 1903, p. 493.

remain quiescent in their Fielde nest[1] for some minutes after the roof-pane had been lifted.

Upon the ants of the other section I practiced such atrocities as that of lifting them frequently by the leg with a pair of forceps or plunging them for an instant in cold water. The ants of this section quickly acquired such associations with the lifting of their roof-pane, that they fled through the compartments of their house in wild panic whenever I touched the glass.

Preliminary Statements.

Is is the purpose of this paper to show that ants have power to recognize certain ant-odors after months or years of separation from those identical odors, and in order that the evidence presented may be plain, it appears necessary to first restate certain facts, well established by past experiments.

1. Ants of different species in different communities or colonies, and also ants of the same species and variety in different communities or colonies, ordinarily show aversion to each other on meeting, and are especially truculent in defense of their young. The cause of this general and perpetual feud among ants of different colonies, is due to difference of odor, discerned through their antennæ, their organs of smell. Fear and hostility are excited in the ant by any ant-odor which she has not individually encountered and found to be compatible with her comfort.

2. If ants of different species, or even of different genera or subfamilies, are made pleasantly acquainted with each other within a few hours after hatching, they will thereafter continue to live together in amity, constituting a mixed colony.[2] The acquaintance thus formed is individual, and every ant, in her later behavior, will act in accordance with individual experience. Acquaintance with the odor of one species or colony does not secure from the experienced ant an amicable reception of a representative of any other species or colony.

Among the mixed colonies, formed by me in August, 1903, twenty *Stenamma fulvum* workers lived with a *Cremastogaster lineolata* queen a full year, and the harmony of the nest was as

[1] Described in Biological Bulletin, Vol. II., No. 2, 1900, and Vol. VII., No. 4, 1904.

[2] "Artificial Mixed Nests of Ants," A. M. Fielde, Biological Bulletin, Vol. V., No. 6, 1903, p. 320.

complete as if its inmates had been of one species. Representatives of three subfamilies, *Formica subsericea*, *Stenamma fulvum*, and *Stigmatomma pallipes* lived amicably together five months before the last Ponerine ant died, leaving the Camponotine and the Myrmicine ants to continue together a full year. *Formica lasiodes*, *Lasius latipes*, *Stenamma fulvum*, *Myrmica rubra* and *Cremastogaster lineolata* affiliated through several weeks. All these and other mixed colonies continued until they were disintegrated by me, and friendly treatment was accorded in them to all introduced ants bearing a familiar and therefore an approved odor, while hostile attack was made on every ant bearing an unfamiliar and therefore a disapproved odor. An enlarged acquaintance with ant-odors did not render any ant tolerant of unknown ant-odors, and in no established mixed colony was an ant of any other than the already represented colonies permitted to live, even when the introduced ant was of the same variety.

I have at the present time a mixed colony of *Camponotus pictus*,[1] *Formica subsericea*, *Formica lasiodes*, and *Stenamma fulvum*, and although there are no young of any species in their nest, they have killed every one of several newly-hatched *Cremastogasters* that I have introduced, and they allow none of the latter species to live when hatched in their nest from introduced pupæ.

3. Ants inherit odor from the queen from whose eggs they are developed. That the queen endows her eggs with an odor, and that newly-hatched queens and workers have an odor recognized by their queen-mother, is proven by the fact that an ant may be isolated from the pupa-stage until it is some days old, never having smelled any ant-odor beside that of its own body, and it will instantly snuggle its queen-mother at first meeting, although it may attack other queens, or sister-workers much older than itself. I have known a young worker to identify its mother among five queens of its species presented for its examination. The queen doubtless recognizes her own odor in the callow that she has never before met.

As I have to present the results of several experiments in which the N colony appears, it will be well to here give some

[1] I am indebted to Dr. William Morton Wheeler for kind identification of *Camponotus herculeanus pictus*, and of *Formica pallide-fulva*. A single ant of the last-named species lived for a year in one of my artificial nests with many *Formica subsericea*.



FIG. 1. *Camponotus pennsylvanicus.* Somewhat magnified. Actual length of queen 2 centimeters.

account of that colony. On July 28, 1903, this N colony of *Camponotus pennsylvanicus* was captured on Nonamesset Island, and was housed in a large Fielde nest. It consisted of a queen two centimeters long, some scores of workers, and numerous cocoons. During the first week in August, 1903, the queen deposited about one hundred eggs, and then ceased laying until the following March. The first larva from the August eggs was observed on August 27. From these larvæ the first cocoon appeared on March 13, 1904. On April 8, 1904, there were many large larvæ in the nest, and there were numerous cocoons varying in length from five millimeters to thirteen millimeters. The first cocoon of this brood hatched on April 24, the temperature of the room being 24° C. or 76° F. These cocoons continued to hatch, most of them in carefully segregated groups, until July 14, when the last cocoon rendered its callow.

Experiment A.—Three large workers hatched each in isolation on July 8, 11 and 14, 1904, from the August eggs of the N queen. On August 5, these three worker-ants, ranging from twenty-two to twenty-eight days in age, and never having met any ant-queen, nor any ant older than themselves, instantly affiliated with their queen-mother, and with each other, at the first meeting. The queen manifestly recognized the odor borne by the callows, and at once snuggled with them.[1] They each recognized in her and in each other the only ant-odor they had ever known, that of their own bodies.

Experiment B. — Four N colony workers, the issue of eggs deposited by the queen in August, 1903, were hatched from segregated cocoons, between May 15 and June 15, 1904. On July 6, when the age of these ants ranged between twenty-one and fifty-two days, and they had never met any other ant of their species, I introduced their queen-mother to their nest. The five immediately affiliated and the previously introduced larvæ were brought and placed beside the queen. The queen must have been at least one year older than these workers, and the workers must have recognized in the queen their own odor at hatching time.

Experiment C. — Five Camponotus workers hatched between

[1] The behavior of the ants in these experiments was observed through an orange-tinted roof-pane, under which the ants behave as if in darkness. See "Supplementary Notes on an Ant," referred to in first foot-note.

April 25 and May 10, 1904, out of cocoons from the August eggs of the N queen. They were from fifty-seven to seventy-two days old, and had never met other ants of their species, when on July 6, 1904, I introduced to their nest their queen-mother. Within five minutes the queen had touched antennæ with every worker and had become the center of a friendly group.

Experiment D. — Fourteen Camponotus workers hatched on or after April 24, and on or before May 10, 1904, out of cocoons from eggs laid by the N queen the preceding August. They had never met other ants of their species, when on July 6, 1904, the oldest of the segregated group was seventy-three days old, and I introduced to their nest their queen-mother. There was for an instant great excitement in the nest, and some tentative nabbing of the queen; but in less than one minute the workers had discovered that she was their own. Within a few minutes, four of the workers had licked the queen, one had stood upon her back, and seven others had grouped themselves close about her.

The behavior of these workers toward their queen indicates that her odor is an unchanging one, or that if there be a change in her odor it is but slowly effected.

The behavior of these ants toward their queen was markedly unlike their behavior toward their sisters, when great diversity in ages was represented.

4. Worker ants change in odor as they advance in age,[1] as was shown by experiments made by me in 1902. Further evidence of this fact will be offered later in this paper. Forty days may pass with no change so great as to elicit from former acquaintances any expression of suspicion or antagonism, but in other cases from forty to sixty days so differentiates the known odor as to inhibit association between the ants.[2] This suspicion

[1] "Notes on an Ant," A. M. Fielde, *Proceedings of the Academy of Natural Sciences of Philadelphia*, December, 1902, p. 609. Also "Cause of Feud Between Ants of the same Species Living in Different Communities," A. M. Fielde, BIOLOGICAL BULLETIN, Vol. V., No. 6, 1903, p. 327.

[2] All the ants employed in the experiments recorded by me have been under my constant care and my frequent observation. No person beside myself has ever had access to them. They have spent the summers, from the first of June to the end of September, at the Marine Biological Laboratory, Woods Holl, Mass., and the remainder of every year in New York City.

or antagonism is always shown[1] by the younger ants, toward the older, if the ants be of the same colony, and if the young ants have been guarded from association with ants older than themselves.[2]

Experiment E. — On July 18, 1904, I put into the nest of two segregated workers of the N colony, hatched from cocoons taken from the wild nest July 28, 1903, and at the time of this ex-

[1] In all experiments here recorded, unless a contrary condition is indicated, the ants whose recognition of a visitor was in question had in their nest inert young that had engaged their attention during several previous days. The action of resident ants toward a visitor is much more prompt and decisive when there are larvæ or pupæ in the nest.

[2] In experimenting with ants, a third source of individual odor, not set forth in this paper, although always reckoned with in the experiments, lies in the other ants with which the individual associates. This odor appears and disappears with certain external conditions. Ants take on the odor of their associates in a mixed nest, and this incidental odor usually disappears after about ten days of isolation, the inherent odor then reasserting itself. Ants may be smeared with the juices of ants of another species or colony and may thereby become immediately subject to attack from comrades from whom they have been but momentarily removed.

I have lately submerged ants for eighty hours or more in distilled water at a temperature of 10° C., putting two species or two colonies into the same water, using thirty-five cubic centimeters for a dozen ants, and I have found that the ants of each species or colony, when revived and returned to their former nest, were attacked as are enemies, and that ten days proved to be an insufficient time for the reassertion of the inherent over the incurred oder. That these attacks from comrades were due to the alien odor acquired in the water and not to some other cause, was shown by the fact that when similar ants were likewise submerged in unmixed groups, the returned ants were amicably received by their former comrades.

I thus submerged, in thirty cubic centimeters of water, three *Camponotus pictus* and fifteen *Stenamma fulvum*, for eighty hours. One of the Camponotus revived, and a day later I returned it to its three former comrades, employed in the care of cocoons in a small Fielde nest. The three instantly attacked the one, and would doubtless have slain it had I not interfered. Having rescued, I isolated it for ten days in a Petri cell, and then again returned it to its former nest. Two of the three resident ants at once attacked and killed it. Of the Stenammas several revived, and on my returning them to their former nest, they were all killed by their quondam associates.

Of four Stenammas that revived and recovered after eight days submergence in company with five *Camponotus pennsylvanicus*, three were killed by former comrades on my returning them to their nest. One of the four was isolated by me, in a Petri cell, for twenty days before I returned her to the nest, and there the returned ant was for some minutes the center of an examining circle of many ants. A day later she was being dragged by two workers, but she was ultimately restored to good standing in the nest.

It is interesting to observe the puzzled or critical demeanor of an ant engaged in ascertaining whether a new-comer has an incurred or an inherent foreign odor.

periment eleven months old, a callow of the same colony, just seven days old. The callow received careful examination from the older ants, and was then amicably entertained by them. They doubtless recognized in the callow their own early odor.

Experiment F. — On July 6, 1904, after the queen and the fourteen workers mentioned in experiment D were all serenely grouped in the nest there described, I introduced two workers[1] of the N colony, hatched between August 14 and September 3, 1903, and therefore about ten months old, while the resident ants were not over seventy-three days old. The two visitors had affiliated previously with the N queen, and had been approved by her. They were probably the issue of her eggs of the previous year. They were, however, seven or eight months older than the resident workers, and, although they were larger than any worker resident, they were persistently attacked and dragged, sometimes by more than one resident at a time. The visitor-ants did not retaliate. One of them tried to placate her young sisters by offers of regurgitated food, and after a half hour there were signs of diminution in the strength of the attacks. I then removed the visitor-ants. There are all degrees in the hostility shown by ants to one another, as well as many variations in the degree of closeness in their affiliations.

In this experiment, the older ants had met no younger ones during their lives, and the younger ones had never before encountered sisters older than themselves.

Recognition of Odors of Other Species.

The A Series.

Evidence of ability to recognize odors that have not been encountered during many months has been taken from ants of diverse species and of a recorded life history. In August, 1903, I formed a mixed colony of workers of *Camponotus pennsylvanicus*, *Formica subsericea* and *Stenamma fulvum*, all of whom hatched in my artificial nests between August 14 and September 3. Every ant within a few hours after its hatching was

[1] My method, which is also Forel's, of marking ants so as to readily distinguish them from others of their species, is described in a foot-note of "Notes on an Ant," already referred to. Ants were marked whenever an experiment required it.

made acquainted with every predecessor in this mixed colony and no discord appeared in the new nest. This mixed colony was marked A. On September 24, when none of these ants was less than twenty days old, and none more than forty-one days old, I separated them according to genera, putting the Camponotus, the Formicas and the Stenammas each into a Fielde nest that was new and therefore had nonestaura. The nests were respectively marked A1, A2 and A3. No young was permitted to hatch in any nest, but inert young from the workers' eggs, or introduced larvæ from other nests were always present when tests of recognition were to be made as severe as possible. No ant of any species was admitted to either of the segregated groups except as recorded in the experiments.

Nest A1. — On April 8, 1904, there were in the Camponotus nest,



FIG. 2. *Stenamma fulvum*, with larvæ and pupæ; slightly magnified. Actual length 5 to 7 mm.

marked A1, three large and vigorous workers, without young. I then introduced two Stenammas that the Camponotus had known in the previous September in the A nest. The Stenammas manifested terror and the Camponotus made instant and violent attack upon them, so that I intervened in the ensuing battle, saved the lives of the Stenammas, and returned them to their own nest. It was evident that during the six and a half months of separation these ants had changed in odor, and that the odor borne by them in April was unknown to the ants with whom they had associated in the previous September. I was unable to offer to this group A1 the companionship of young Stanammas having the same odor as had their former associates at the time of association, and young Stenammas taken at a later date from the wild nest in the summer of 1904, were always killed by them. The Formicas were likewise rejected.

Nest A2. — The Formicas that had lived, none less than twenty and none more than forty-one days, in nest A, were domiciled in nest A2. They had been separated from the Camponotus six and a half months, when on April 8, 1904, I introduced into their nest, where there were twenty-six workers and no young, a Camponotus from nest A1, comrade of their earliest days. The Formicas immediately attacked the Camponotus, and I removed the latter to save her life. Six month's progress in odor formation had carried her outside the acquaintance of her former associates. The same antagonism was manifested toward the other two residents in nest A1.

These Formicas continued to reside in their nest and had laid a few eggs, which were under their care when on April 25, 1904, I introduced to their nest three Camponotus newly hatched from eggs that were laid in August, 1903, by the queen-mother of the Camponotus in nest A1. These young Camponotus received amiable welcome from the resident Formicas, and during the next ensuing days, to May 10, I added several more callows. The Formicas, now eight months old, continued to live amicably with the young Camponotus, whose odor had been known to them in their earliest days. They regurgitated food to the young ants, permitted them to carry the egg-packet and care for the larvæ, and in all respects treated them as if of their own colony. There

was no death in the nest for twenty days after the introduction of the young Camponotus, but I removed them on June 25. *The Formicas recognized, after seven months of separation from it, an ant-odor previously known to them.*

I was unable to introduce to this nest any young Stenammas that the Formicas would accept, as I could command none of the same lineage and age as those known to them in the autumn of 1903. The individual Stenammas in nest A3, with whom they had formerly associated in the A nest, were now of another odor, and the Formicas refused to affiliate with them.

Nest A3. — The Stenammas in this nest had lived in nest A, none less than twenty and none more than forty-one days, and since September 24, 1903, they had met no ant other than those in their own nest. On April 8, 1904, I introduced into their nest, where, there were forty-one workers, a former comrade, a Formica from nest A2. She was fiercely attacked, and I removed her. Other Formicas from the same nest were likewise attacked. I then introduced Camponotus from nest A1, and they were received with like animosity. It was instantly made evident that the resident Stenammas found in every visitor an unfamiliar odor. I removed each visitor as soon as her status among these Stenammas had been made plain, and the A3 nest remained quiescent until April 24, when I introduced a day-old Camponotus, the issue of an egg laid the previous August by the mother of the rejected individual in nest A1. Within a few minutes after its introduction, three of the Stenammas had licked the Camponotus, and all the Stenammas had viewed it with approval. It was taken care of as tenderly as if it had been a Stenamma callow. On the same day I added two newly hatched Camponotus of the same lineage, and they were kindly entertained until April 27, when I removed them to another nest of Stenammas, the M nest. These M Stenammas were of the same age and colony as were the Stenammas in nest A3, differing from them only in never having lived with Camponotus. There were also about the same number of resident ants. As soon as I introduced the Camponotus into the M nest, the Stenammas attacked them, and although they were double the size of the residents, and of tougher integument, the residents harried them to death. The



FIG. 3. *Lasius latipes*, magnified. Actual length 5 millimeters.

behavior of the M ants indicates that that of the A3 ants was based on a recognition of odor, and that *the Stenamma's power of recognition extends through an interval of at least seven months.*

The B Series.

Another series, marked B, was also established, having its beginning on July 20, 1903, additions of newly-hatched workers being made to the mixed colony up to August 28, or during a period of forty days. The B mixed colony consisted of *Stenamma fulvum* of the C colony, *Lasius latipes* of the F colony, and *Cremastogaster lineolata* of the H colony. On September 24, 1903, I separated these ants according to genera, segregating each genus in a new Fielde nest, marked B1, or B2 or B3, where it remained until the end of the recorded experiments, with no other ants than those here mentioned ever introduced or permitted to hatch in its nest.

Nest B 1. — On April 1, 1904, over six months after their segregation, there were in the B1 nest about sixty Stenammas. I then introduced to their nest two Lasius, their former comrades, taken from nest B2. No fear was evinced by the Lasius, and no aversion by the Stenammas. Residents and visitors perfectly affiliated, and the two Lasius remained safely in the B1 nest four full days. I then, on April 5, removed the two Lasius, and put them into a nest of *Stenamma fulvum* where there were two queens and sixteen workers, all hatched in August, 1902, and therefore a year older than were the ants in nest B1. They were from the same C colony, but had never associated with Lasius. In this nest the Lasius tried to flee or to hide, behaving as do ants when in a nest of recognized enemies. At first they eluded the Stenammas, but when I again examined the nest, on the evening of the same day, both Lasius had been killed and put on the rubbish-heap.

It appears that these very strong-smelling ants, the Lasius, had not so changed their odor during six months that the B1 Stenammas did not recognize it. But on June 11, 1904, I took from the wild nest of the F colony two Lasius workers, of unknown age, and introduced them into the B1 nest of Stenammas. They were soon killed, and two like them were also killed when introduced on the ensuing day.

On August 11, 1904, I introduced a newly hatched Lasius from the R colony, and it was at once killed.

On June 11, 1904, I sought in the old wild nest of the Lasius F colony for a remainder of its population, and secured a few workers and four larvæ. On August 4, three cocoons and one naked pupa had appeared from these larvæ, and from these cocoons the first worker hatched on August 18. It was immediately put into the B1 nest, where it appeared as a yellow pigmy among brown giants. It was much patted with the antennæ, was licked, and was cared for among the eggs, larvæ and pupæ over which the Stenammas were strenuously engaged. *The Stenammas recognized an odor from which they had been eleven months separated.*

The Stenammas of nest B1 met no Cremastogaster from September 24, 1903, until July 7, 1903, when I introduced into their

nest, occupied by fifty of the old residents, with larvæ from their own eggs, several newly-hatched *Cremastogaster lineolata* from a wild nest V. Within a day all these young Cremastogasters had been killed and their bodies piled together in a corner of the food-room. Others of their kind met a like fate on July 9.

On the 24th of June, I had happily secured some larvæ, with workers to rear them, from the wild nest of the old H colony of Cremastogasters, and on July 22, I put a dozen callows, newly hatched from this stock, into the B1 nest, where the residents were much engrossed with their own pupæ. Two or three of these introduced callows were killed and dismembered, while all the rest were accepted into close companionship by the resident Stenammas. The Cremastogasters were permitted to walk over or rest upon the pupa-pile, they were gently licked, and none was harmed during the ensuing twelve days. I then removed them to prepare the nest for another experiment.

But while the Cremastogasters were still in the B1 nest, on July 25, I introduced a newly hatched *Formica lasiodes*, in order to see whether these Stenammas would accept a callow of unknown odor. This visitor was immediately killed and carried to the rubbish-pile.

Further evidence that the Cremastogasters were offspring of the queen that laid the eggs from which the B3 Cremastogasters issued, was obtained by putting some of them into K nest with an H colony queen that had never before met any Cremastogaster workers of any colony, having spent her whole life since she was hatched in August, 1903, with Stenamma workers. This queen and the Stenamma workers with her, all accepted the H colony Cremastogaster workers hatched in the latter part of July, 1904, and continued to closely affiliate with them. They were doubtless of the same odor as was the queen in this K nest, being progeny of the same queen in different years, the queen in K nest still retaining her queen-mother's odor, while the worker-callows bore the queen-mother's ordor as yet unchanged by ageing.

Stenamma fulvum in my artificial nests, when they had no young of their own, have many times permitted Cremastogaster pupæ to hatch in their nest and to live there among them. But

when engaged in the care of their own young, they have, unless previously acquainted with Cremastogasters, always killed the Cremastogasters as soon as the latter hatched and began to move about. It appears that the Stenammas of nest B1 recognized an old acquaintance in the young Cremastogasters of the H colony, and resumed toward them their accustomed behavior. In other words *the Stenammas recognized an odor after an interval of ten months in which that odor had not been encountered.*



FIG. 4. *Cremastogaster lineolata;* magnified. Actual length 5 millimeters.

Having removed all Cremastogasters from nest B1 on August 4, there followed an interval of twelve days in which they had met no Cremastogasters. Then on August 16, 1904, I introduced to their nest one of their old comrades in nest B, a Cremastogaster from whom they were separated on September 24, 1903. This visitor was killed by them within a few hours. In less than eleven months she had attained an odor unknown to them.

Nest B2. — There were in April, 1904, so few of the Lasius in this nest that I gave their power of recognition no test within their own nest. All of them were used till their extermination, in the experiments in nests B1 and B3.

Nest B3. — On April 5, 1904, over six months after the segregation of the Cremastogasters, there were about fifty workers in their nest, B3. I then introduced two Lasius, with whom they had lived in amity some forty days, and from whom they had been separated over six months. The residents attacked the visitors from nest B2, and would have slain them had I not rescued them. There was no room for doubt concerning the absence of recognition among the Cremastogasters of the present odor of the Lasius, ants presenting an odor so strong that a single one of them is very impressive to human nostrils. I was unable at that time to introduce to these Cremastogasters any *Lasius latipes* younger than were those with whom they had formerly associated. But on August 12, 1904, when I introduced a newly-hatched *Lasius latipes* of the X colony, they immediately killed it. On August 20, 1904, I introduced a young Lasius from the wild nest of the F colony, a sister of the one put on the 18th into nest B1. This ant was amiably received. *The Cremastogasters recognized an odor from which they had been eleven months separated.* On the same day, August 20, 1904, I also introduced an adult *Lasius*, of unknown age, but also of the F colony, one of the nurses of the newly-hatched ant already accepted. This adult *Lasius* was killed during the ensuing night.

On April 8, 1904, I introduced to these Cremastogasters, in nest B3, two of the Stenammas with whom they had pleasantly lived for forty days, in the preceeding autumn, and from whom they had been separated more than six months. One of the visitors behaved as if in an alien nest, showing fear and attempting to escape. The other fought with a resident. Absence of recognition on either side indicted such change of odor during the period of separation as to render these ants unacquainted with one another.

Early in July, 1904, I was able to introduce to this nest several newly-hatched Stenammas from the wild nest of the C colony, kindred of the ants in nest B1. There were many queens in that wild nest. That the callows might not bear the odor of older ants, I segregated some pupæ, and offered the callows newly hatched therefrom to the ants in nest B3, but these callows were all killed and dismembered within a few days after

their introduction. They were doubtless the progeny of other queens than those which produced the early acquaintances of the Cremastogasters in nest B3.

Hypothesis.

From such data as is here presented, correlated with records of past years, it is possible to diagramatically represent the probabilities that an ant, isolated from the pupa-stage, would encounter a known odor at a first meeting with another ant of her colony. If the odor of a queen be unchanging, if she impart odor to all her eggs, if that odor be perceptible in the inert young, and if from the beginning of the active life of the worker there be a progressive change in the inherited odor borne by her, then from each summer's deposit of the queen's eggs there would be in the following summer more than one odor among the workers, because all the eggs of a queen are not hatched during the summer in which they are deposited. We may suppose a young, fertilized queen, the founder of a colony, to deposit eggs in her isolated cell in July, and to have reared her first small brood in not less than sixty days.[1] The eggs laid by her in the latter part of summer or early autumn would reach the larval stage in late autumn, and in that stage would be carried over to the next June, to hatch as ants in summer. While this second brood was developing, the first brood would have advanced to the odor of ants many months old. Their odor would then be unknown to an ant newly hatched from the queen-mother's egg and having the odor of its own body as its only criterion of ant-odor. Succeeding years would bring similar conditions.

In Sir John Lubbock's nests, one ant-queen lived to her fourteenth, and another to her fifteenth year; but the purpose of the diagram is reached by the supposition that the queen lives ten years.

[1] For time of incubation of eggs, larval period and pupa-stage in *Stenamma fulvum*, see "A Study of an Ant," A. M. Fielde, *Proceedings of the Academy of Sciences of Philadelphia*, September, 1901, p. 430. The time of incubation appears to be twenty days for all ants that I have observed; the larval period may be extended to at least one hundred and forty days in a high temperature, and probably to a much longer time in cold weather; and the pupa-stage occupies about twenty days. I have known the larval period to be passed in twenty days.

Diagrammatic representation of the progressive change of odor in worker-ants.

The arabic numerals, in horizontal line, indicate successive years in the life of a queen, the founder of a colony.

The Roman numerals at the left hand, denote successive broods from the eggs of this queen, here supposed to be the only fertile female of the colony.

The letters are used as symbols of the odor of worker-ants of this colony.

	1	2	3	4	5	6	7	8	9	10
I	*a*	*b*+*a*	*c*+*b*	*d*+*c*	*e*+*d*	*f*+*e*	*g*+*f*	*h*+*g*	*i*+*h*	*j*+*i*
II		*a*	*b*+*a*	*c*+*b*	*d*+*c*	*e*+*d*	*f*+*e*	*g*+*f*	*h*+*g*	*i*+*h*	*j*+*i*
III			*a*	*b*+*a*	*c*+*b*	*d*+*c*	*e*+*d*	*f*+*e*	*g*+*f*	*h*+*g*	*i*+*h*	*j*+*i*
IV				*a*	*b*+*a*	*c*+*b*	*d*+*c*	*e*+*d*	*f*+*e*	*g*+*f*	*h*+*g*	*i*+*h*	*j*+*i*
V					*a*	*b*+*a*	*c*+*b*	*d*+*c*	*e*+*d*	*f*+*e*	*g*+*f*	*h*+*g*	*i*+*h*	*j*+*i*
VI						*a*	*b*+*a*	*c*+*b*	*d*+*c*	*e*+*b*	*f*+*e*	*g*+*f*	*h*+*g*	*i*+*h*	*j*+*i*
VII							*a*	*b*+*a*	*c*+*b*	*d*+*c*	*e*+*d*	*f*+*e*	*g*+*f*	*h*+*g*	*i*+*h*	*j*+*i*

If all the ants from this queen's seventh year eggs and having the *a* odor were segregated from their pupa stage, onward, the only ants of this colony with which they would affiliate on meeting would be such ants as were hatched in her seventh year from eggs laid by the qeeen in her sixth year. If groups of such ants were segregated they would, if hatched less than forty days apart, affiliate on meeting.

Ants of the *b*, *c*, *d*, *e*, *f* and *g* odors would affiliate with ants of the *a* odor, because that odor was once familiar to them in their own bodies. It would be a recognized odor.

The conditions of odor in her colony during her lifetime are crudely and only approximately represented by the diagram, but the representation conduces to an understanding of certain phenomena observed by me in the C colony, which I have now studied four years, in its natural nest, and in my artificial ones.

When queens, instead of flying away to found new colonies, remain in the colony where they hatched and increase its population by their progeny, there is opportunity for all the members of that colony to receive a liberal education of the chemical sense. Every ant acts on individual experience, and if its experience be narrow it will quarrel with many, while acquaintance with a great number of ant-odors will cause it to live peaceably with ants of diverse lineage, provided the odors characterizing such lineage and age environ it at its hatching. If some of the workers were separated from their colony in their youth, and kept segregated several years, the sequestered ants could amicably meet younger ants from their old home nest only by an act of memory, or a power of recognition spanning the interval of their separation from their colony. Precisely this condition has been created by me with ants of the C colony.

The C colony is a great community of *Stenamma fulvum* that lives under stones scattered at considerable intervals over an area ninety yards in diameter, along a lane and pasture. I have been unable to find any other colony of Stenammas in its vicinage. Many of its young queens appear to mate with their kin and remain in the colony. I have found as many as fourteen deälated queens in a single shovelful of its nest-earth. The queens and workers from its extreme limits always affiliate unhesitatingly on meeting within its domain.

On August 22, 1901, I took from under a central stone of this colony queens, males and workers, and divided them into two sections, each of which was kept segregated in a Fielde nest for two years. No young was permitted to hatch in either section, and when I united the two sections in August, 1903, they affiliated instantly, and also affiliated less perfectly with queens and workers freshly brought from the wild nest.[1] I kept the ants of

[1] Detailed account of these meetings may be found in "Cause of Feud between Ants of the Same Species," already referred to, p. 328.

the two united sections, again without young, another year, until August, 1904, when I introduced to their nest marked queens and workers from their old wild nest. Of the two queens introduced one was at once received into full fellowship. The other was simultaneously licked and dragged by different worker-residents, but was accepted at the pupa-pile within a few hours. The workers introduced had kindly reception. Of one hundred and fourteen callows introduced one by one, or a few at a time, an interval of repose being given between the removal of one and the introduction of its successor, only two were attacked. While it is true that all the accepted callows of the summer of 1904 might have had the odor of the resident queen, it appears more probable that most of them *bore odors that were recognized by the resident workers after the lapse of three years.*

These ants had certainly not within their three years of segregation met a two-year-old or a one-year-old ant of their colony, from outside their artificial nest. In August, 1902, I segregated a queen and workers all hatched during that month, and in August, 1903, I likewise segregated a newly hatched and similar group. All these ants were of the C colony, and no young was permitted to hatch in any of these groups, each in its artificial nest. In August, 1904, I therefore had command of ants of the C colony, one group in the C nest, consisting of ants brought in from the wild nest on June 24, 1904; one group C1, just one year old, one group C2, just two years old, and one group C3, three years old or more. All had been acquainted with their seniors before segregation. In August, 1904, the three-year-old ants received amiably, within their nest, ten of the two-year-old ants, and ten of the one-year-old ants, indicating a perfect recognition of odors no longer represented in their own nest, and from which they had been long separated.

On the other hand, when I introduced the three-year-old ants, queens or workers, into a nest populated with hundreds of workers taken in June, 1904, from the wild nest or hatched within that nest during the present summer, the three-year-olds were always fiercely attacked. *They had become an alien colony to the younger generations of their former wild nest.* The C colony has been much harried in its natural domain, by the rebuilding

of stone fences, by repairing of the lane-road, and by my own depredations. Ants so old as my three-year-olds have apparently become almost unknown in the wild colony.

The older the colony, the fewer would be the chances that any ant, segregated as a pupa and always kept in isolation, would find its own odor in any ant taken at random from the nest from which said pupa had been taken. In August, 1904, I thus isolated pupæ and the ants hatched therefrom, and from among many experiments made with them, I record the following as typical: I isolated a pupa from the C colony wild nest, and when the ant that hatched from it was eleven days old, never having smelled any ant-odor other than that of its own body, I introduced one by one to its Petri cell, where it was engrossed in the care of introduced larvæ, all the three-year-old ants to the number of twelve, all the two-year-old ants to the number of seventeen, all the one-year-old ants to the number of forty-two, and seven C nest ants of precisely its own age, so that the number of visitors arriving singly and at intervals amounted to eighty. A period of repose was provided after the removal of one visitor before another was introduced. Every one of these visitors was at first meeting violently attacked by this callow, dragged away from the larvæ, and in some cases taken outside the Petri cell, if I lifted its cover. Sometimes a visitor violently attacked the resident callow, and she had to be rescued by me from sudden death. In other like series of experiments with isolated callows the resident callow sometimes found a congenial odor in a visitor and willingly permitted her to share in the care of the larvæ.

Callows having their origin in different parts of the C colony area behaved alike, and it appears improbable that all of those tested could have been the product of eggs, larvæ or pupæ brought in by raids on another colony, especially when the greatness of the C colony is considered and its location studied.

Other experiments with callows from this colony gave support to my hypotheses. On July 11, 1904, there hatched in nest C3, a pupa previously introduced by me from the C nest. I left it seven days with the three-year-old ants, and then transferred it to a Petri cell, giving it a few larvæ to care for. This isolated callow knew only its own odor, that of worker ants at least three

years old, and that of a queen. I then, a day or two later, introduced into its cell, one by one, all the one-year-old ants in nest C1, removing each visitor as soon as the action of the resident was decisive, and allowing a period of repose before another visitor was introduced. She affiliated with the first, third, fourth, sixth, seventh, eighth, tenth and eleventh, and attacked the second, fifth and ninth.

I likewise introduced two-year-old workers from nest C2. She affiliated with the first, third, fourth, fifth, seventh, ninth and twelfth, and attacked the second, sixth, eighth, tenth and eleventh. She did not attack the C2 queen, but the queen so persistently avoided her as to make the test undecisive.

In the same manner I tested a callow, hatched on July 11, in nest C2, where all the ants beside herself were two years old. When this callow had spent seven days in the C2 nest, and one day in isolation with larvæ to care for, I introduced into her cell workers from the C3 nest, where all the ants were three or more years old. She affiliated with the second, fourth, fifth, eighth, eleventh and twelfth, and attacked the first, third, sixth, seventh, ninth and tenth visitors. I then introduced one-year-old ants from nest C1. She affiliated with the first, second, third, seventh, eighth, ninth, tenth and twelfth, and attacked the fourth, fifth, sixth and eleventh visitors.

I intended to likewise test a callow reared in nest C1, and I expected to find that this callow would reject all three-year-old ants; but I unfortunately dropped an unmarked three-year-old ant into nest C1, and thereby so vitiated the nest as to make it useless for this experiment.

A comparison of all the tests made gave a consensus of testimony that the C3 ants, *the Stenammas, recognized and adapted their behavior to ant-odors that they had not encountered during three years.*

As the workers are not supposed to reproduce colonies, and as the queens are not supposed to change their own odor, how then would queens of diverse odor originate through the ageing of the workers?

In 1901 I segregated winged queens of the C colony,[1] putting

[1] "Notes on an Ant," previously referred to, p. 605.

some of them into nests with kings of their own colony and others of them into nests with kings of alien colonies, and believed that I ascertained that the progeny of sister queens affiliated, regardless of paternal influence in the egg from which the ants issued. I then supposed that queen-ants captured before swarming must be virgin queens. I now know that virgin queen-ants often mate with the males within the maternal domicile, and that neither the possession of wings nor an early capture guarantee the virginity of a queen. Only by sequestration of the queen from her pupa-stage can her virginity be secured. I have had *Lasius latipes* queens drop their wings and lay eggs soon after being brought from the wild nest from which they had not yet swarmed. In my artificial nests, I have observed the persistent avoidance, by queens, of kings of alien colonies and their manifest preference for kings of their own colony. Mating in captivity, in artificial nests, is not uncommon, and it must be frequent in the wild nests before the swarming.

We know that the eggs of workers often produce sturdy males, and it appears probable that such males impart to the fertilized eggs of the queen something of the odor attained by the worker-mother at the time when the egg, producing the male, was deposited. This would differentiate odors in the progeny of sister queens, and cumulative differentiation would account for ultimate differences in the odor of queens of the same species and variety. When queens remain in the mother-nest after mating, and there rear their broods, that colony must become one of much mixed odors, as is the C colony described in this paper. Fertilized queens, departing from the maternal nest, would found colonies whose issuing queens would have an odor depending on the age of the workers who were mothers of kings hatched in the season in which their founder-queens mated.

Besides discerning the aura of the nest and other local scents and the track laid down by its feet,[1] an ant perceives in other ants the *incurred* or incidental odor which appears with conditions and disappears in course of time; the *inherited* odor derived from the queen-mother, apparent in the eggs, larvæ, pupæ and newly

[1] "Further Study of an Ant," A. M. Fielde, *Proceedings of the Academy of Natural Sciences of Philadelphia*, November, 1901, p. 521.

hatched young, and probably strengthening as size increases through the three inert stages of development; the *progressive* odor, that distinguishes the worker and changes or intensifies with her advancing age; and the *specific* odor which pertains to the species or tribe. Adding to these perceptions the power of recognizing familiar odors after a lapse of months or years, the ant appears to be well equipped for life in her world.[1] If she has not reason and imagination, she has at least the ground on which to exercise both, cognoscence of past experiences.

[1] The organ discerning the nest-aura and probably other local odors lies in the final joint of the antenna, and such odors are discerned through the air; the progressive odor or the incurred odor is discerned by contact, through the penultimate joint; the scent of the track, by the antepenultimate joint, through the air; the odor of the inert young, and probably that of the queen also, by contact, through the two joints above or proximal to those last mentioned; while the next above these by contact also discerns the specific odor. It is probable that the size of the queen determines the amount of odor diffused by her. The amount of odor diffused by or discerned in the larvæ and pupæ may be the determining factor in the assorting of the young according to size, as is common among ants. The results of many experiments whereby the function of many joints in the antennæ were determined by me in 1901–1903 in *Stenamma fulvum* are recorded in "Further Study of an Ant" and "Cause of Feud among Ants of the same Species," above referred to. The joints in the antennæ vary in different species, from four to thirteen.

NOTES ON A HITHERTO UNDESCRIBED HYDROID FROM LONG ISLAND SOUND.

CHAS. W. HARGITT.[1]

Early during the present year I received from Dr. Henry R. Linville, of New York City, a small collection of hydroids for examination, among which was found what clearly appears to be a new species. The specimens were collected by Dr. Linville during the previous August near East Marion, Long Island. In a letter to the writer he describes the habitat as follows :

"The species was found growing on rocks and piles under 'Milldam Bridge,' west of Shelter Island. The bridge spans a narrow creek which connects the Bay with a shallow salt-water pond."

In size and general features the hydroid resembles very much *Syncoryne mirabilis*, and was at first thought to be that species. A more critical examination showed this to be more than doubtful, as the number of tentacles, character of the hydranth, and above all the character of gonophores, all indicated otherwise, and a review of the accessible literature failed to afford any clue to the identity of the species, though it seems clearly a member of the genus above named as will be seen from the sketch.

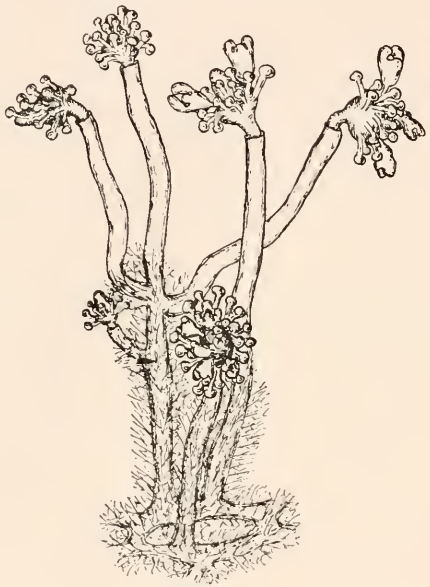
The medusa-buds are relatively large and grow in clusters of from two to four upon the body of the hydranths usually close among the tentacles. I regret that no free medusæ were obtained and that only an incomplete description of this organism can be made. As will be observed from the figure the medusæ present the somewhat unusual condition of having at this stage two of the tentacles quite well developed, though short and thick, at opposite positions on the margin of the umbrella. The intermediate pair can hardly be distinguished, being mere buds, and it is impossible to say whether they later develop or not; indeed, it remains doubtful as to the later phases of development of almost all the medusoid organs, though there can hardly be serious doubt as to the ultimate freedom of the medusæ, as there were

[1] Contributions from the Zoölogical Laboratory, Syracuse University.

no signs of the development of gonads, a condition almost certain to have been found in case the gonophores remain sessile.

The following diagnostic characters will serve to distinguish the species, at least so far as the hydroid phase is concerned, for which the name *Syncoryne linvillei* is proposed:

Trophosome. — Colony growing in tufts, sparingly branched, to a height of 15 to 30 mm., and with the same general aspects as characterize *S. mirabilis* Ag. Hydranths vasiform, with cone-



Portion of colony of *Syncoryne linvillei* about three times natural size.

shaped proboscis; tentacles definitely capitate, from 15 to 30 in number, and variously distributed over the proximal third of the hydranth body. Perisarc much as in *S. mirabilis*, plain, or with only the slightest trace of any annulation, ending somewhat abruptly below the base of hydranths, proximal or basal portion dense or brownish in color. Hydrorhiza more or less reticulated, forming a loose network over the substratum. In all the type specimens both hydrorhiza and hydrocaulus were enmeshed in a dense sponge-like mass, though its exact nature is doubtful.

Gonosome. — Medusa buds borne on body of hydranth, usually in small clusters among the bases of the tentacles and supported by a single peduncle, the terminal specimen always maturing

first. In the type specimens no free medusæ were present, though there were numerous buds approaching full development. The umbrella is distinctly bell-shaped, with four radial canals; tentacles unequally developed, two on opposite sides large and club-shaped, the intermediate pair small and bud-like. No ocelli distinguishable.

Colors. — Formalin material pale yellowish, hydranths somewhat brownish; gonophores reddish brown or pink.

Habitat. — Growing in tufts upon small rock fragments, bits of bark or on shelly concretions; the bases of stems tangled or enmeshed in sponge-like masses, the tufts and spicules of which give to the colonies a rough and bristly aspect.

A SYNOPSIS OF CHARACTERS OF SOME FISHES BELONGING TO THE ORDER HAPLOMI.

EDWIN CHAPIN STARKS.

The orders Haplomi and Iniomi are distinguished from the Nemotognathi (the cat fishes) and the Plectospondyli (the minnows and suckers) by having normal anterior vertebræ; from the Isospondyli (the herring and trout-like fishes) by the absence of a mesocoracoid; from the several orders of eel-like fishes, by the comparatively few vertebræ, the presence of ventral fins, and the unrestricted gill openings; from several peculiar small orders by apparently sufficient characters, but which for present purposes need not be considered.

Thus these orders seem to be made up of soft-rayed fishes, which are thrown together because they lack the distinguishing characters of other groups, rather than because they have such characters of their own. This condition naturally would tend to bring together forms that are not very closely related.

The families of the Haplomi have either widely diverged from each other or are not of the same line of descent. The order is not held together by any important character, though some very peculiar characters may be used to rather widely separate three groups.

I have not studied the Iniomi,[1] but from the definition given below I find it impossible to separate the Haplomi from it. The free condition of the scapular arch has been the most useful character in separating these two orders, but as this condition is found in the families Esocidæ and Umbridæ, it cannot be used unless we transfer these families from the latter order to the former. Obviously this is not advisable as *Umbra*, especially, is certainly nearer the family Pœciliidæ than it is to any family in the order Iniomi.

The Iniomi was established by Dr. Gill [2] to include those forms

[1] Except to determine that the condition of the attachment of the scapular arch to the cranium is the same in *Synodus* as it is in *Esox* and *Umbra*.

[2] Proceedings of the U. S. National Museum, 1884, p. 350.

in which the scapular arches are "connected with and impinge on the occiput behind and on each other, and are otherwise free from the cranium." In his original definition he calls Iniomi a group, and later in his "Families and Subfamilies of Fishes" [1] he treats it as a superfamily. Jordan and Evermann in "Fishes of North and Middle America" raise it to ordinal rank, and point out that the absence of the mesocoracoid is the most important character by which the Iniomi are separated from the Isospondyli.

A readjustment of the orders Haplomi and Iniomi will be necessary, and either a separation based on characters unstudied and unconsidered, or a merging of one into the other will result. This will necessitate a study of many more forms than are here considered.

The knowledge of the following characters I obtained in studying the relationships of *Dallia pectoralis*. As I am not at this time ready to take up an extensive investigation of the orders Haplomi and Iniomi it seems better, while it is fresh in my mind, to publish this material in the following form as a working basis, rather than to hold it indefinitely in abeyance.

Order HAPLOMI.

Soft rayed fishes without a mesocoracoid and with the anterior vertebræ normal. Parietals separated by the supraoccipital. Alisphenoids not meeting in a median line in front of brain case. Exoccipitals separated by basioccipital. Postclavicle composed of a single element. Actinosts four. Opercular bones all present. Ventral fins abdominal, each attached to a simple flat pelvic bone. Pectoral fins placed low. Dorsal fin placed more or less posteriorly. Air bladder with a distinct pneumatic duct.

Superfamily Esocoidea.

Ethmoid represented by paired elongate dermal bones; metapterygoid present, forming the upper margin of the cheek bones above the symplectic; symplectic normal; palatine and pterygoid both present and normal; the former with teeth; primaxillaries

[1] Sixth Memoir, National Academy of Sciences, Vol. VI.

without backward extending processes on median line of snout; maxillaries forming lateral margin of mouth; two toothed and two toothless superior pharyngeals present on each side; post-temporal connected to the epiotic by a ligament;[1] nearly meeting its opposite fellow just behind the occipital region; supra-clavicle normal in size; post-clavicle a single ray of bone; hypercoracoid foramen only notching the lower edge of hypercoracoid;[2] actinosts rather elongate, all ending against hypocoracoid or in cartilage opposite that bone; pelvic bones without an overlapping spur; upper end of shoulder girdle attached by ligament to first vertebra; parapophyses normally developed only posteriorly; the hæmal spines which support caudal attached by suture to their centra; posterior vertebræ with a very decided upward bend;[3] vent normal in position.

Family ESOCIDÆ.

Characters as indicated by *Esox reticulatus.*

Cranium long and slender, with a narrow projecting rostrum; interorbital septum single; myodome short not opening posteriorly; the prootic shelf above not nearly reaching to mouth of anterior opening to brain case; parietals extending over a deep cavern to pterotic; suborbital present; a lateral wing extending upward from parasphenoid barely reaching to alisphenoid; septomaxillaries[4] present between ethmoid and vomer; vomer large, reaching anterior to parasphenoid; nasals present; basisphenoid extending upward from parasphenoid, Y-shaped and unattached above; opisthotic absent; palatine joined to prefrontal by a slender process; anteriorly without a process hooking over maxillary; a wide open space between the hyomandibular and the preopercle opposite the middle of the latter; premaxillaries widely separated from each other by the rostrum; maxillary with a

[1] In a specimen of *Esox* 40 cm. in length the ligament is 4 mm. long. In *Umbra* it is comparatively as long.

[2] See exception under description of Esocidæ.

[3] In *Esox* the narrow hypural plate is placed at an angle of about 45 degrees, and the hæmal spine from the preceding vertebra runs horizontally back to the base of the middle caudal rays.

[4] E. P. Allis, Jr., discusses the septomaxillaries and the paired ethmoid of *Esox*, in a paper entitled "On Certain of the Bones of the Cheek and Snout of *Amia calva*," *Jour. of Morph.*, Vol. XIV., No. 3, 1898.

large supplemental bone; a large preorbital and four suborbitals present; posttemporal a wide plate bent longitudinally at a right angle; its posterior edge concave, not typically forked; its lower limb connected by ligament to exoccipital; a broad ribbon of connective tissue connecting the inner surface of the supraclavicle with the first vertebra;[1] hypercoracoid simply notched by its foramen, which opens against cartilage between the coracoid elements;[2] lower pharyngeals elongate, not in contact at their inner edges; an ankylosed epipleural on each of the two first vertebræ; vertebræ number 36 + 17 = 53; all abdominal neural processes attached by suture; parapophyses developed as processes only posteriorly.[3]

Family UMBRIDÆ.

Characters as indicated by *Umbra lima*.

Cranium short and normal in shape; interorbital septum double, with the sides widely separated throughout; no myodome; parietal separated from pterotic by an area of cartilage; supraorbital absent; parasphenoid not sending a lateral wing up to alisphenoid; vomer very small, scarcely larger than its patch of three or four teeth; loosely attached to the surface of the parasphenoid, and nowhere reaching to the edge of that bone; no septomaxillaries or nasals present; a preorbital but no suborbitals present;[1] basisphenoid absent; a very small loosely attached opisthotic present in usual position;[2] palatine with a process hooking over the maxillary; no open space between

[1] The connecting band does not appear to be of the same compact ligamentous nature as in *Umbra*, but is composed or more loosely connected fibers. It does not join the tip of a lateral process, but is attached directly to the side of the vertebra.

[2] In a specimen examined of *Esox lucius*, about the same size as the above (40 cm. in length) the foramen is entirely contained by the hypercoracoid.

[3] The parapophyses are represented by small ossicles attached to the centra by suture, and set in sockets so that the ribs appear to be attached directly to the vertebræ until careful examination is made. They first appear on the third vertebra, but are little developed in front of the ninth or tenth. Posterior to the twenty-eighth they develop outward and downward as small processes in front of the base of each rib. Posteriorly they grow longer and the last two or three are ankylosed to the centra.

[4] The sensory tunnel which is usually continued from the frontals by the nasals, opens to the exterior as soon as it leaves the former bone and extends no further forward.

[5] It is easily pulled away with the posttemporal, remaining attached to the lower ligament of that bone.

the preopercle and the hyomandibular; premaxillaries meeting at the median line on snout as usual; no supplemental maxillary bone; lower limb of posttemporal represented by a short triangular process from which a ligament runs to the opisthotic;[1] a strong ligament extends from a lateral process on the first vertebra to the inner surface of the supraclavicle;[2] the hypercoracoid foramen is a notch in the hypercoracoid open against the hypocoracoid; lower pharyngeals in contact along their inner edges; vertebræ number 21 + 14 = 35; epipleurals of first vertebræ not ankylosed; all neural processes ankylosed to vertebral centra; no parapophyses developed except on last four abdominal vertebræ;[3] ribs fitting into depressions with slightly raised edges on the body of the vertebræ; the first vertebra carries no rib.

Superfamily Pœciloidea.

Ethmoid represented by a single nearly circular scale of bone; metapterygoid absent; the symplectic forming the upper margin of the cheek bones behind the mesopterygoid; symplectic very large, sending a long slender process behind the quadrate nearly to the condyle of the mandible; palatine-pterygoid arch reduced to a single element, and without teeth;[4] premaxillaries each with a small backward extending process on median line of snout; premaxillaries forming lateral margin of mouth; superior pharyngeals either three toothed patches, or ankylosed into a single one on each side; the usual toothless one of first arch either present or absent in the former condition, but that of second arch never toothless; posttemporal directly attached to epiotic without the intervention of a ligament; supraclavicle a very small scale of bone, scarcely sufficient to separate the posttem-

[1] Four specimens have been examined and no indication found towards ossification of this ligament as in *Dallia*.

[2] This ligament is probably the homolog of the ray of bone in *Dallia pectoralis* that is in the same position.

[3] The process on the first vertebra to which the supraclavicle ligament is attached may possibly be considered a parapophysis also.

[4] The posterior end of the palatine-pterygoid element borders the upper anterior edge of the quadrate, and is braced above by the mesopterygoid, as is usual for the pterygoid; the anterior end is attached to the prefrontal and maxillary, as is usual for the palatine; making it appear probable that these two elements have ankylosed.

poral from the clavicle; postclavicle a simple ovate scale of bone; hypercoracoid foramen entirely enclosed by the hypercoracoid; actinosts small, deeper than long, no opening between them, two on each the hypercorcoid and the hypocoracoid; pelvic bones each with a spur extending inward, one of which overlies the other; upper end of shoulder girdle joined to bassioccipital by a long ligament;[1] large projecting parapopyhyses present on all abdominal vertebræ; the caudal supporting hæmal spines ankylosed to their centra; the posterior vertebræ not tilted up; vent normal in position.

FAMILY PŒCILIIDÆ.

Subfamily FUNDULINÆ.

Characters as indicated by *Fundulus similis*.[1]

Interorbital septum double, its sides widely separated throughout; no myodome; parasphenoid sending a lateral process up to alisphenoid; supraoccipital expanded latterly on top in a thin horizontal wing; epiotic developed backward in a long thin process as in the genus *Mugil;* occipital condyle partly formed by exoccipitals; prefrontals not meeting at the median line; basisphenoid and opisthotic absent; mandible normal; premaxillaries normally curved; posttemporal a simple bone with no indication of a lower fork, normally attached to epiotic; three superior pharyngeals on each side joined (not ankylosed) to form an ovate plate, covered with molar-like teeth near middle of bone, which change to small blunt conical teeth near outer edges; lower pharyngeals joined to each other by deeply dentate and interlocked suture,[3] together forming a triangular bone with concave

[1] The ligament runs from the middle of the outer edge of the basioccipital to the upper end of the clavicle in *Pœcilia* and *Cyprinodon*, equally to the inner surface of the supraclavicle and the posttemporal in *Fundulus*.

[2] *Gambusia* (as exhibited in species *nobilis*) agrees with *Fundulus* in character of superior pharyngeals and unforked posttemporal but in the condition of the occipital condyle resembles *Pœcilia*.

[3] The lower pharyngeals of the following species of *Fundulus* have been examined: *siminolis*, *catenatus*, *majalis*, *stellifer*, *diaphanus*, *nottii*, *notatus*, *sciadicus*, *rathbuni*, *zebrinus* and *heteroclitus*, and a nearly uniform gradation found from the first, where the pharyngeals were joined by simple suture, together forming a triangular plate, to the last, where they were elongate and attached only at their anterior ends, diverging posteriorly. They were all joined more or less loosely. None were attached by dentate suture, as in *similis*. The gradation from one to another was in about in the order here given.

sides, covered with teeth similar to those above; interhyal present; urohyal laterally expanded, broader than high; parapophyses present on all abdominal vertebræ, the posterior ones the largest, much smaller than in *Pœcilia*, but otherwise similar.

Subfamily Cyprinodontinæ.

Characters as indicated by *Cyprinodon carpio*.

Interorbital septum single; a short wide myodome developed; parasphenoid not sending a lateral process up to alisphenoid; supraoccipital crest normal; occipital condyle partly formed by exoccipitals; prefrontals meeting at the median line; a small basisphenoid extending upward from the parasphenoid, its upper end unattached; opisthotic absent; mandible and premaxillaries normal; posttemporal widely forked, its upper limb normally joined to epiotic, its lower limb to exoccipital at some distance from the opisthotic region,[1] without the intervention of a ligament; superior pharyngeals as in *Fundulus*, the teeth sharper, none of them molar; lower pharyngeals loosely joined to each other, together forming a triangular-shaped bone; interhyal present; urohyal normal; parapophyses as in *Fundulus*.

Subfamily Pœciliinæ.

Characters as indicated by *Pœcilia elongata*.

Interorbital septum double, the sides widely separated throughout; no myodome; parasphenoid sending a lateral process up to alisphenoid; supraoccipital crest expanded laterally on top in a thin horizontal wing; epiotics not produced backwards; occipital condyle confined to basioccipital; prefrontals not meeting at the median line; basisphenoid absent; a small opisthotic present; mandible reduced in size, its elements of complex shape, and loosely connected; side of maxillary turning at a sharp angle with the front; posttemporal forked, the upper limb firmly attached to the cranium, lying closely against the oblique upper surface of the epiotic for nearly its whole length, and leaving scarcely any space between its base and the skull; the lower fork attached directly to the opisthotic without the intervention

[1] The opisthotic when present in other species covers the suture between the exoccipital and the pterotic.

of a ligament; superior pharyngeals ankylosed into a single elongate bone on each side, and covered with about sixty rows of very fine close set bristle-like teeth, which grow larger posteriorly; lower pharyngeals large, joined along their entire inner edge (not ankylosed) to form a single concave surface, curved to coincide with the convex superior pharyngeals, covered with teeth similar to those above, but longer and more bristle-like anteriorly; interhyal absent; other hyoid bones normal; very large parapophyses present on all abdominal vertebræ, growing larger to the fourth pair, thence smaller posteriorly; none of them transversely connected; the ribs attached to their tips.

Superfamily Amblyopsoidea.

Ethmoid an unpaired median bone; metapterygoid present above a normal symplectic; palatine and pterygoid both present; the former with teeth; premaxillaries with small backward extending processes; three tooth bearing superior pharyngeals present on each side; posttemporal forked and normally attached to cranium, the upper limb directly to epiotic, the lower by a short ligament to the opisthotic; supraclavicle well developed; postclavicle absent; hypercoracoid entirely containing its foramen; actinosts as in the Pœciliidæ; upper end of clavicle attached to first vertebra by a ligament; pelvic girdle composed of two small simple rays tapering forward and apparently not in contact with each other; ventral fins sometimes absent; parapophyses present on all abdominal vertebræ; the caudal supporting hæmal spines ankylosed to their centra; posterior vertebræ not tilted up; vent close behind isthmus.

Family AMBLYOPSIDÆ.

Characters as indicated by *Amblyopsis spelæus*.

Interorbital septum double, the sides widely separated; no myodome; no lateral process from parasphenoid to alisphenoid, but the latter developed downward to the side of the former largely enclosing the side of the cranium anterior to the prootic; no supraoccipital crest developed; occipital condyle partly formed by the exoccipitals, which are widely separated by the basioccipital; opisthotic well developed; parasphenoid very wide, ante-

riorly bending up at the sides and leaving only a very small space between it and the frontal; three tooth bearing superior pharyngeals present on each side; not connected to form a single patch; the first very small, the last two very much larger and equal in size; lower pharyngeals loosely in contact at the median line; the last basibranchial bearing teeth similar to those on the lower pharyngeals; hyoid bones all present and normal; vertebræ number $14 + 16 = 30$; neural and hæmal spines all ankylosed to the vertebral centra; the spines of only two vertebræ anterior to the hypural assist in supporting the caudal.

Stanford University.

Vol. VII. *November, 1904.* *No. 6*

BIOLOGICAL BULLETIN

FORM-REGULATION IN CERIANTHUS, VII.

Tentacle-Reduction and Other Experiments.

C. M. CHILD.

ERRATA.

Vol. VII., No. 3, page 167. Fig. 1. A *normal* skeleton of an anterior forearm and hand. Fig. 2. The skeleton of a *regenerated* hand.

It is not necessary to give the data of the experiments in full since they add nothing essential to the facts already given for *C. solitarius*. A few points are, however, of some interest especially in view of certain experiments which Loeb performed on this species.

In consequence of the greater thickness and stiffness of the body-wall in this species as compared with *C. solitarius* it is much less difficult to obtain pieces in which the enteron remains more or less widely open for a considerable length of time, in some cases indefinitely. In such pieces tentacle-regeneration is greatly delayed but sooner or later some regeneration does occur.

Loeb ('91, pp. 44 and 45, Figs. 4–6) describes briefly and figures cases of this kind which he obtained by cutting rectangular pieces from one side of the body. His figures show that regeneration of the tentacles occurs but they also show that it is much delayed, since in his Fig. 6, the most advanced stage shown,

riorly bending up at the sides and leaving only a very small space between it and the frontal; three tooth bearing superior pharyngeals present on each side; not connected to form a single patch; the first very small, the last two very much larger and equal in size; lower pharyngeals loosely in contact at the median line; the last basibranchial bearing teeth similar to those on the lower pharyngeals; hyoid bones all present and normal; vertebræ number $14 + 16 = 30$; neural and hæmal spines all ankylosed to the vertebral centra; the spines of only two vertebræ anterior [illegible] supporting the caudal

BIOLOGICAL BULLETIN

FORM-REGULATION IN CERIANTHUS, VII.

Tentacle-Reduction and Other Experiments.

C. M. CHILD.

Experiments on Cerianthus Membranaceus.

In the preceding papers certain experiments upon *C. membranaceus* have been mentioned from time to time. It was impossible on account of lack of material to make my studies of this species as full and complete as those of *C. solitarius*. The data obtained were sufficient, however, to show a close correspondence between the two species as regards regulative phenomena.

It was possible to delay tentacle-regeneration by preventing the establishment of internal water-pressure by the methods employed for *C. solitarius*, viz., by reopening at short intervals or by giving the pieces a form such that closure was delayed or prevented.

It is not necessary to give the data of the experiments in full since they add nothing essential to the facts already given for *C. solitarius*. A few points are, however, of some interest especially in view of certain experiments which Loeb performed on this species.

In consequence of the greater thickness and stiffness of the body-wall in this species as compared with *C. solitarius* it is much less difficult to obtain pieces in which the enteron remains more or less widely open for a considerable length of time, in some cases indefinitely. In such pieces tentacle-regeneration is greatly delayed but sooner or later some regeneration does occur.

Loeb ('91, pp. 44 and 45, Figs. 4–6) describes briefly and figures cases of this kind which he obtained by cutting rectangular pieces from one side of the body. His figures show that regeneration of the tentacles occurs but they also show that it is much delayed, since in his Fig. 6, the most advanced stage shown,

the tentacles are only a few millimeters in length, although this piece is two months older than Fig. 4. A piece regenerating in the typical manner during more than two months should possess marginal tentacles 25–40 mm. in length.

Loeb's figures and text indicate that little attention was paid to the internal anatomy of the form. He states that in these rectangular pieces the number of tentacles regenerated was a fraction of the whole number possessed by the original animal corresponding to the fraction of the total circumference represented by the oral end of the rectangular piece, but no reference is made to the correspondence of the tentacles with the intermesenterial chambers. According to Loeb's statements and figures the tentacles appear to arise from the oral cut surface of the piece in each case, but according to my own observations they never arise in this manner. It is difficult to understand how hollow, tubular tentacles could arise directly from the cut edge of the body-wall. This point is of some importance, since if Loeb is correct the influence of internal pressure upon the regeneration of the tentacles becomes at least extremely doubtful.

In the first paper of this series ('03*a*) the typical course of regeneration in *C. solitarius* was described. It was shown there that the new tentacles do not arise at the cut surface but at some little distance from it. The margin of the body-wall rolls inward after section and the tentacles arise at the highest, most oral part of the rounded margin thus formed ('03*a*, Figs. 13–19).

In the specimens of *C. membranaceus* which I have observed the tentacles arise in exactly the same manner. The margin rolls inward and soon changes in the body-wall appear in a region several millimeters distant from the actual cut surface, viz., at the most oral region of the inrolled part. Thus an area extending about the whole circumference is established in which first loss of pigment and reduction in thickness of the body-wall occur, and then the new marginal tentacles appear as minute buds.

In the open pieces corresponding to Loeb's Figs. 4–6 the tentacles arise in exactly the same manner, though much more slowly than in closed pieces. The margin of the piece rolls inward and the new tentacles appear on a part of the body-wall which was originally somewhat aboral to the cut surface.

At first glance it may appear that the regeneration of tentacles in these pieces with open enteron cannot be influenced in any way by internal pressure, but a brief consideration of the matter will be sufficient, I think, to show that even in these pieces some degree of internal pressure is possible. It must be remembered first that the circulatory currents running orally in the intermesenterial chambers continue in pieces with open enteron, except where contraction may bring about the more or less complete obliteration of certain chambers by approximation of the mesenteries. These currents force the water orally into the inrolled region at the oral end. If now the exit of the water from this region be hindered in any way, some slight degree of distention may arise within this rolled portion. Repeated examination of pieces of this kind has led me to the conclusion that the inrolled edge usually presses against the free margins of the mesenteries with some force and may thus hinder in some degree the exit of water. The diagrams 1 and 2 will serve to illustrate this point. In these figures a longitudinal section of the oral end is shown, the cross-hatched portion representing the mesenteries. In Fig. 1 the inrolled edge of the body-wall is shown. Since these pieces represent only a part of the whole circumference of the body the inrolling at the oral end is usually greater than in a complete cylindrical piece, for in the latter the inrolling about the whole circumference soon results in mutual pressure between the various parts of the inrolled portion which prevents further inrolling. The result of the inrolling is to press the cut surface against the margins of the mesenteries. In the collapsed condition these folded and thickened margins form a dense mass and the inrolling of the cut edge upon this must press them still more closely together. In whatever spaces remain between the mesenteries the cilia force the water orally as indicated by the arrows in Figs. 1 and 2. The margins of the mesenteries, being more or less closely pressed together, do not readily permit the escape of the water and consequently a certain degree of pressure may be maintained. Diffusion of water through the walls in this region may also aid in maintaining pressure.



Fig. 1.



Fig. 2.

According to this view these cases are merely special cases of more or less complete local closure of the enteron by inrolling. Various experiments of similar kind have already been described (Child, '04*c*). The only difference between those and these is that here the inrolling is transverse across the oral end and consequently permits the appearance of tentacles upon the whole inrolled portion. Fig. 2 shows the condition of the end after the regeneration of tentacles has begun. By this time the mesenteries in the region of the inrolled margin have acquired new relations to the body-wall such that the margin is held in position. The reduction in thickness of the wall in the upper portion of the inrolled region represents the beginning of tentacle formation. In every case of this kind which I have observed conditions have been similar. The only conclusion possible is that Loeb's statement that the tentacles arise from the cut surface is incorrect and that in the pieces shown in his Figs. 4–6 the tentacles arose in the manner which I have described.

If my explanation is correct, then the results obtained from these open pieces do not contradict the conclusions of my previous papers, viz., that internal water-pressure constitutes a factor in tentacle-regeneration. As regards local pressure due to circulatory currents and its possible effects the statements made in previous papers (Child, '04*b*, '04*c*, '04*d*) will apply here.

In all pieces of this kind the appearance of the tentacles is much delayed and their regeneration is very slow. In no case observed do they ever attain more than a small fraction of the normal length. They are, moreover, always more or less blunt and frequently very irregular, *i. e.*, of different lengths. Similar characteristics were described and discussed for certain cases in *C. solitarius* in a previous paper (Child, 04*c*). Thus in *C. membranaceus* as in *C. solitarius* the facts indicate that internal pressure plays an important rôle in tentacle-regeneration.

The Reduction of Normal Tentacles.

Not only was it possible to retard or inhibit the regeneration of tentacles by reduction of the internal water-pressure, but it was found that fully developed tentacles could be reduced in length by reducing the internal pressure. This process of re-

duction comprises two distinct stages. During the first stage the collapsed tentacles gradually become shorter but do not change in appearance, except to become more and more deeply colored as the pigment-particles are more and more closely approximated with reduction in surface. This reduction continues during a month or more according to various conditions, *e. g.*, the completeness of collapse, the temperature, etc.

If, however, the condition of collapse be continued during a still longer period the tips of the tentacles began to atrophy, becoming darker in color and shrivelled or withered in appearance. This process of atrophy gradually extends proximally along the tentacle if the internal pressure is not reëstablished, until in some cases mere stumps of tentacles remain. The Figs. 3–5 show



FIG. 3.

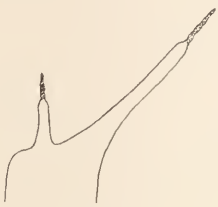


FIG. 4.



FIG. 5.

schematically the process of atrophy, one side of the disc with tentacles being shown in section in each figure. Fig. 3 represents a stage in which the marginal tentacles have been reduced by about one half their length and the labial tentacles relatively less. Figs. 4 and 5 represent later stages. In Fig. 5 the long shrivelled distal portion seen in Fig. 4 has been almost completely resorbed. The reduction in size of the disc in consequence of reduced pressure is also indicated in the figures. The difference in appearance between the healthy and shrivelled portions is always marked and visible at a glance. The tips are always much darker in color than the normal tentacle and are irregular in contour, so that the use of the term "shrivelled" is amply justified by their appearance. The proximal healthy portions are about equal in length in all the tentacles of each set, *i. e.*, the atrophy of the tips proceeds with equal rapidity in all tentacles of each set. The tips apparently always remain closed.

A gradual reduction in size of the shriveled portions, due to slow resorption, occurs, and these parts may disappear almost completely, leaving blunt, stump-like tentacles with only slight traces of the dark atrophied tissue at their tips (Fig. 5).

A few experimental data will serve to illustrate more fully this process of atrophy and the conditions under which it occurs.

Series 50.

November 21, 1902. — In three specimens of *C. solitarius* the aboral end was removed by a transverse cut (Fig. 6) and the aboral half of the distal piece was split longitudinally by several irregular cuts in order to render closure impossible or difficult. In these pieces the aboral end was reopened daily or on alternate days in the manner described in a previous paper (Child, '04*b*).

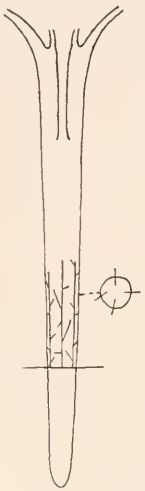


FIG. 6.

Controls were obtained by removing the aboral end from three specimens at the same level as in the experimental pieces. These controls were allowed to remain undisturbed and the cut ends closed in the usual manner. In all specimens, both experimental pieces and controls, the tentacles were measured in a fully distended condition before section. The marginal tentacles were 15–20 mm. in length, differing somewhat in different specimens; the labial tentacles were 5–7 mm.

December 17, 1902. — 26 days after section. Experimental pieces. Reopened daily on alternate days, but sometimes became more or less distended during the intervals, owing to approximation of the cut margins and plugging of the intervening spaces with slime. During this time the tentacles have gradually decreased in length. Marginal tentacles 6–8 mm.; labial tentacles 3 mm.

Controls. The tentacles remained collapsed for a day or two after section, then, as closure of the ends proceeded, the internal water pressure was established and the tentacles became distended. The length of the tentacles is about the same as before section, viz., marginal tentacles 15–20 mm.; labial tentacles 5–7 mm.

From this time on the three experimental pieces were left undisturbed in order to determine whether, after closure of their aboral ends and distension, the tentacles would regain their original length.

December 28. — 37 days after section. Experimental pieces. All healed aborally and fully distended with water. During the eleven days since December 17 the tentacles gradually elongated. In the three pieces the lengths of the tentacles were as follows: marginal tentacles, 12–15 mm., 11–12 mm., 10 mm.; labial tentacles, 5 mm., 4 mm., 3–4 mm.

The longest tentacles at this time belong to the piece which originally possessed the longest tentacles, viz., marginal, 20 mm., labial, 7 mm.

Controls. — In no case do the tentacles show any appreciable reduction in length.

No further increase in length of the tentacles of the experimental pieces occurred.

During the first 26 days the marginal tentacles of the experimental pieces decreased more than 50 per cent. They were more or less completely collapsed during this period, and as a collapsed tentacle is always shorter than one that is distended, it may appear at first glance that the reduction in length represents merely this difference. This, however, is not the case. During the first few days following section the collapsed tentacles were only slightly shorter than they had been when distended and a continuous gradual reduction in length took place up to December 17. During the following period of eleven days in which closure and renewed distension occurred, the tentacles elongated gradually. If the reduction in length had represented only the difference between a collapsed and a distended tentacle it might be expected that the tentacles would regain their original length immediately or almost immediately after the internal pressure and distension were again established. But the increase in length was gradual, although the tentacles were apparently fully distended about December 21. Moreover, they never regained their original length, the maximum being 70 per cent. of the original length.

These facts render it evident that an actual reduction in the

length of the tentacles occurred during the period of reduced water-pressure. The failure of the tentacles to regain their original length is probably due to the loss of vigor observable in all specimens kept without food for a long period and the consequent diminution in internal water-pressure and in the power of growth. Regenerated tentacles never attain the length of the original tentacles and similarly those tentacles elongating after reduction never attain the original length.

In this experiment the process of reduction did not pass beyond the first stage. The tips of the tentacles did not shrivel, but remained healthy and became distended again after closure occurred, though the original length was never attained. It is possible that the frequent partial distension of the tentacles during the intervals between reopening of the aboral end was sufficient to prevent the death of the tissues. As was noted in a previous paper ('04*b*) the pieces must be reopened every few hours in order to prevent partial distension in the intervals, but this is impracticable for the long time necessary for perfect results. Consequently the method is by no means perfect.

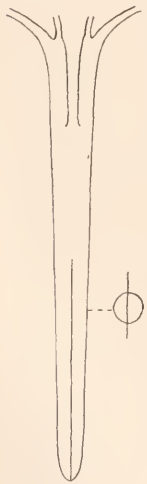


FIG. 7.

Series 19.

September 15, 1903. — In six large specimens the aboral half of the body was split by a longitudinal cut into two parts (Fig. 7).

The object of this experiment was not tentacle reduction but the duplication of the aboral end and consequently the lengths of the tentacles were not determined as frequently during the earlier stages as in experiments on tentacle-reduction. Later, however, when it was observed that these specimens would afford interesting evidence upon this point, measurements of the marginal tentacles were made regularly.

The split aboral portions of these specimens rolled in various ways and closure was greatly delayed, the delay being further increased by the frequent rupture of the delicate new tissue in consequence of contraction during the necessary examinations.

The length of the tentacles in the fully distended specimens before section was as follows: marginal tentacles 25–30 mm.; labial tentacles 12–13 mm.

After section the usual collapse occurred and the tentacles gradually decreased in length.

October 6. — 21 days after section. The pieces have remained almost completely collapsed since section. In some instances a part of the body which was more or less completely closed off became slightly distended, but, the frequent examination and removal of slime caused contraction and total collapse. The tentacles were not measured, but marked reduction had occurred during the period of collapse and at this time the tips of all the marginal tentacles were beginning to atrophy.

October 22. — 37 days after section. Two pieces were lost during the interval. The remaining four pieces were somewhat distended with water but any contraction caused loss of water either in consequence of rupture of new tissue or through one or both of the duplicated aboral pores about which the regenerating muscles had not fully developed. Consequently the internal pressure was still relatively very slight. Extensive atrophy of the distal portion of both marginal and labial tentacles had occurred. The marginal tentacles measured 6–7 mm. + 1–2 mm. of shrivelled tissue representing the distal portion of the tentacle. The labial tentacles measured 3–4 mm. + 1 mm. of shrivelled tissue. The basal healthy portions of the tentacles ended bluntly instead of in slender tips as in normal specimens: upon these blunt ends were borne the small shrivelled masses of tissue.

During the following month the pieces succeeded in closing sufficiently to permit considerable increase of internal pressure and no longer lost water during slight contractions.

December 1. — 77 days after section. In three of the pieces the marginal tentacles have increased in length to 10 mm. and the labial tentacles to about 5 mm.

In the fourth specimen the marginal tentacles were 7–8 mm. and the labial tentacles about as before.

In all the atrophied tips have almost completely disappeared, apparently having undergone resorption, but the tentacles are still blunt, not tapering like normal tentacles.

During the next twenty days the pieces showed a considerable reduction in size in consequence of decreasing internal pressure and the distal portions of the tentacles once more began to shrivel.

December 21. — 97 days after section. Marginal tentacles in all four pieces 3–4 mm. + 1–2 mm. of shrivelled tissue.

It was evident at this time that the pieces were becoming exhausted and the experiment was concluded.

In these pieces a gradual reduction in the length of the tentacles occurred during the first two weeks, then the tips began to atrophy, thus reducing the functional portion of the tentacle still further. When closure of the pieces permitted distension the tentacles elongated again and the atrophied tips underwent almost complete resorption. Still later, as the pieces became exhausted and the temperature of the water became lower, the tips of the tentacles once more began to shrivel and at the conclusion of the experiment mere stumps remained.

Reduction of the tentacles was also observed in *C. solitarius* in many œsophageal pieces, *i. e.*, pieces retaining the tentacles and with the aboral end in the œsophageal region (Fig. 8). In such pieces the œsophagus united with the body-wall aborally as in the pieces described in the preceding paper ('04*d*), and except in cases where closure of the body-wall across the end of the œsophagus occured, the internal pressure was never reëstablished.

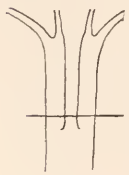


FIG. 8.

In one piece of this kind which closed aborally across the end of the œsophagus after eleven days the marginal tentacles decreased in length before closure from 25–30 mm. to 10–12 mm. and the labial tentacles from 10–12 mm. to 6–7 mm. After closure the length of the marginal tentacles increased again to about 20 mm. and that of the labial tentacles to about 10 mm. Here the period of collapse was comparatively short and there was no shrivelling of the tips of the tentacles. In another œsophageal piece the marginal tentacles decreased in three months from 35 mm. to mere stumps 3 mm. in length and the labial tentacles from 12 mm. to 1.5 mm. In this case, as in Series 19 above, the reduction of the tentacles consisted at first

merely of a decrease in length, the tissues remaining normal in appearance except as regards the darker color due to the collapsed condition. Later shrivelling of the tips began and continued until only blunt rounded stumps bearing small masses of the dark, shrivelled tissue remained.

In *C. membranaceus* a similar reduction of tentacles was observed, both in pieces which were kept open by artificial means and in œsophageal pieces, but in no case did the distal portions shrivel as in *C. solitarius*. This difference in behavior is probably due to the firmer consistency of the tissues of *C. membranaceus*. In one piece which was opened every day during forty-nine days the marginal tentacles decreased from 40–50 mm. to 10–15 mm. and the labial tentacles from 20 mm. to 3–4 mm. This piece was then allowed to close and become distended and during the next thirty-nine days the marginal tentacles increased in length to 20 mm. and the labial tentacles to 5 mm. In this case the decrease in length was nearly 75 per cent. for the marginal tentacles and about 80 per cent. for the labial tentacles. The marginal tentacles after renewed distension attained nearly 50 per cent. of the original length, the labial tentacles only 25 per cent.

The œsophageal pieces of *C. membranaceus* were not observed to close aborally across the end of the œsophagus in any case. It was therefore impossible to observe increase in length of the tentacles after reduction in these pieces, but marked reduction was observed in all cases. In one piece the marginal tentacles decreased during twenty-seven days from 60 mm. to 20–25 mm. and the labial tentacles from 25 mm. to 10–12 mm. and in others a similar reduction was noted.

It is possible that atrophy of the tips of the tentacles would have occurred if the specimens had been kept for a still longer time. On the other hand this process may be so slow in this species that it is visible merely as a gradual reduction in size.

In view of these facts there can be little doubt that the length of the tentacles is influenced by the internal pressure. The question as to whether it is the general internal pressure which exerts this influence, the effect being merely more conspicuous in the tentacles than elsewhere, or whether local pressure due to currents plays a part must be decided by future experiments.

Various other facts noted in the course of my experiments require mention here. It was noted that regenerating tentacles attained a greater length during the summer months than during winter (*cf.* Child, '03*b*). This difference is probably due, at least in part, to the fact that the internal pressure is higher in summer than in winter in consequence of the greater activity of the cilia in forcing water in through the œsophagus (*cf.* Child, '04*b*). Local pressure must also vary in a similar manner.

It was also observed in a large number of cases that the tentacles of normal animals or regenerated specimens of *C. solitarius* often began to shrivel at the tips in the manner described above when the specimens had been kept in captivity and without special feeding for two months or more. Frequently the marginal tentacles were reduced to a length of 5–6 mm., the specimens appearing otherwise healthy and in good condition, except that the body was in all cases less distended than in newly taken specimens. In the light of the preceding experiments the conclusion is justifiable that the tentacle-reduction in these species is due to decrease in internal water-pressure. The gradual exhaustion of the pieces and the consequent diminution of ciliary activity is without doubt one factor in the result.

As winter approached it was noted that the atrophy of the tentacles began sooner and proceeded farther. During December many of the specimens brought into the laboratory from the bay possessed marginal tentacles only 8–10 mm. in length with atrophied tips and labial tentacles similarly reduced. In nearly all specimens with the tentacles normal in appearance at the time of taking, the atrophy of the tips began within a week or two.

In January and the early part of February, beyond which time my observations do not extend, more than a hundred specimens were taken and of these about 75 per cent. possessed reduced tentacles with atrophied tips when taken. Many of the remainder were specimens with regenerating tentacles, which had undoubtedly been injured.

During the summer and autumn reduced tentacles with atrophied tips were not found on any specimen at the time of taking. Moreover, the tentacles of summer and autumn specimens were

always much longer than those of winter specimens even when the latter were not atrophied.

These facts leave no room for doubt that the reduction of the tentacles and the loss of their tips is a normal phenomenon during the winter months. I was able, however, to bring about this change experimentally in September and October, *i. e.*, at a time when newly taken specimens showed no trace of such a change.

Unfortunately it was impossible to continue observations during the spring. I have no doubt, however, that as the temperature of the water rose the internal pressure became greater and the tentacles increased in length again.

The animals do not thrive in aquaria with standing water and it was not practicable to supply the aquaria with running water kept at a constant temperature approaching that of the water in summer. For these reasons the experiment of keeping the animals with reduced tentacles in water of a higher temperature than that in which they had been living could not be carried out satisfactorily. I venture to predict, however, that the result of such an experiment would be an elongation of the reduced tentacles, though probably they would never attain the full summer length unless the animals were well fed.

There is no ground for supposing that the specimens with atrophied tentacles were in a pathological condition. Specimens which were in this condition when taken lived for months in captivity and regenerated as completely as other specimens. After atrophy of a certain portion of a tentacle the atrophied portion was gradually absorbed and the remaining proximal portion retained a perfectly healthy appearance until the gradual decrease in internal pressure due to exhaustion brought about a further atrophy. These reduced tentacles could be distinguished at once from normal tentacles by their blunt tips.

The only conclusion possible in view of the facts appears to me to be that any condition which reduces the internal water-pressure to a sufficient extent will bring about the atrophy of the tentacles. Experimentally reduction of pressure was accomplished by preventing closure, but it is clear that any condition which decreases ciliary activity will decrease the internal pres-

sure, since the force of the inward current through the œsophagus is reduced. The force of circulatory currents in the body must be similary reduced and consequently any local pressure due to them is also reduced.

Decrease in temperature is a condition which reduces ciliary activity. It follows that in winter specimens the cilia must be much less active than in summer specimens. Winter specimens are visibly less distended than summer specimens. I can see no escape from the conclusion that the atrophy of the tentacles in winter specimens is due as in experimental pieces to a long-continued reduction to a relatively low level of the internal water-pressure, resulting in this case from the reduction of ciliary activity in consequence of low temperature.

The fact that the shrivelling of the tentacle always begins at the tip and proceeds gradually in the proximal direction is of some importance. If this process is the result of decrease in the general internal pressure why should the reduction proceed from the tip? According to the laws of hydrostatics the internal pressure must be the same in all parts of the enteric cavity, consequently when reduction of the pressure occurs it occurs at all points. This being the case why should a slight reduction of pressure affect merely the tip of the tentacle, while a greater reduction causes atrophy of a larger part?

In the present state of our knowledge two explanations of this fact appear possible. It may be that the distal portions of the tentacle exist under relatively unfavorable conditions of nutrition as compared with the proximal portions. The small size of the tentacular cavity in this region and the consequent imperfect circulation of fluid and also its distance from the central cavity where most of the digestion probably occurs, afford a possible basis for such an assumption. If the stimulus of internal pressure is effective upon the tissues of *Cerianthus* we should expect to find the poorly nourished parts of the body more sensitive than other portions to decrease in this stimulus. According to this view reduction of internal pressure might result in atrophy beginning at the tips of the tentacles and proceeding proximally according to the degree and duration of the reduction.

The second explanation is based upon the possible effect of local pressure due to circulatory currents. It is evident that with approach toward the distal end of the tentacle the circulatory currents must decrease in force and volume since in the confined space of the tentacular cavity the friction between the currents in opposite directions is relatively greater than in the enteron. The force and volume of the currents must also depend upon the degree of distension of the tentacle and body and therefore upon the general internal pressure. If, therefore, the internal pressure be reduced, the tentacular cavity decreases in size, and the circulatory currents are practically eliminated in the extreme distal portion of the tentacles: local pressure at the tip of the tentacle is thus reduced. It is possible that this is a factor in bringing about a condition of malnutrition. According to this view the atrophy of the distal portion may be the result of the reduction of local pressure in the distal portion of the tentacle in consequence of the reduction in force and volume of the circulatory current. The larger, proximal portion is still of sufficient size even under reduced pressure to permit the existence of these currents.

Since I have mentioned in previous papers certain facts which seem to indicate the possibility that local pressure due to circulatory currents may constitute a formative factor in *Cerianthus* the possible effect of these currents is mentioned in this connection. It is possible that both reduction in local pressure and malnutrition of the distal portions of the tentacles may coöperate in causing atrophy under reduced general pressure. But whatever interpretation may be placed upon the facts, the occurrence of atrophy under reduced internal pressure is clearly shown.

Summary.

1. Pieces of *C. membranaceus* in which the enteron remains widely open to the exterior may show some degree of tentacle-regeneration. In such pieces the inrolling of the oral margin and the pressure of the cut surface against the mesenteries may be sufficient to prevent the escape of water and thus permit the establishment of some degree of internal pressure within the in-

rolled portion. In all cases of this kind tentacle-regeneration is much delayed and never proceeds far.

2. It is possible by reduction of the internal water-pressure to cause reduction of fully grown normal tentacles in both *C. solitarius* and *C. membranaceus*. The first stage in the reduction consists in a gradual decrease in length and size of the tentacles: in *C. membranaceus* no further change was observed, but in *C. solitarius* shrivelling and atrophy of the tentacles began at the tips if the reduced pressure continued. In specimens which were kept widely open the tentacles were reduced to mere stumps a few millimeters in length.

3. If specimens with partially atrophied tentacles be allowed to close, the basal healthy portion of the tentacle begins to extend as the internal pressure is reëstablished.

4. Atrophy of the distal portion of the tentacles occurs in specimens of *C. solitarius* which are kept for a long time without food and is also found in nearly all specimens taken during the midwinter season.

5. Since the internal pressure in normal animals is due primarily to the activity of cilia any conditions which reduce the activity of the cilia reduce the pressure. Low temperature and starvation or exhaustion cause reduction in ciliary activity and so in internal pressure. If these conditions continue for a certain length of time atrophy begins at the tips of the tentacles, proceeding proximally as the reduction in pressure continues.

6. The fact that atrophy due to reduced pressure always begins at the tip of the tentacle may be explained by the relatively greater reduction in the force and volume of the circulatory currents in the distal region in consequence of the smaller size of the tentacular cavity in that region or it may be due to a condition of malnutrition in the distal regions of the tentacles which in turn is the result of the small size of the tentacular cavity and the distance from the main digestive cavity. In this condition the distal regions of the tentacle are less capable of continuing their existence under unfavorable conditions (*e. g.*, reduced pressure) than the more proximal portions. Possibly both of these factors are concerned in the result.

Hull Zoölogical Laboratory,
University of Chicago, November, 1903.

BIBLIOGRAPHY.

Child, C. M.

'03a Form-Regulation in Cerianthus, I. The Typical Course of Regeneration. Biol. Bull., Vol. V., No. 5, 1903.

'03b Form-Regulation in Cerianthus, II. The Effect of Position, Size and Other Factors upon Regeneration. Biol. Bull., Vol. V., No. 6, Vol. VI., No. 1, 1903.

'04a Form-Regulation in Cerianthus, III. The Initiation of Regeneration. Biol. Bull., Vol. VI., No. 2, 1904.

'04b Form-Regulation in Cerianthus, IV. The Rôle of Water-Pressure in Regeneration. Biol. Bull., Vol. VI., No. 6, 1904.

'04c Form-Regulation in Cerianthus, V. The Rôle of Water-Pressure in Regeneration; Further Experiments. Biol. Bull., Vol. VII., No. 3, 1904.

'04d Form-Regulation in Cerianthus, VI. Certain Special Cases of Regulation and their Relation to Internal Pressure. Biol. Bull., Vol. VII., No. 4, 1904.

Loeb, J.

'91 Untersuchungen zur physiologischen Morphologie der Thiere. I. Ueber Heteromorphose. Würzburg, 1891.

LOCOMOTION IN BATRACHOSEPS WITH SEVERED NERVE-CORD.

CHARLES D. SNYDER.

The earlier discoveries in the anatomy and physiology of the nervous system led to the conception that the nervous mechanisms of the human body were under the control of a single center, the brain.[1] Discoveries, which have been made in the field of comparative physiology during the past fifty years, however, are lead-to a very different conception.

Not only the spinal cord,[2] but also the brain,[3] is being regarded not as a single nerve-center but as a segmental series of many nerve-centers. Furthermore the idea is gaining ground that true nerve-centers are probably not so greatly differentiated one from the other, — that their respective protoplasmic contents are not so different in nature, as was once supposed. On the other hand it is pointed out that specific differences in the nervous substance is to be looked for in the sense-organs, in the peripheral nerve-substance, rather than in the central organs. Accordingly the central nervous system is to be regarded primarily as a special conduction substance by means of which the sense organs are able to communicate with the various physiological structures in which the efferent nerve-fibers find their termination.[4]

The observations reported in the present paper furnish additional evidence in support of this segmental theory of the central nervous system. In order to make the viewpoint of interpretation of results clearer the work of two other authors will be first briefly reviewed.

Friedländer[5] observed that coördination of the longitudinal

[1] Schiff, "Lehrbuch der Physiologie," 1858, s. 194. Foster, "History of Physiology," 1901, p. 257 ff. Steiner, "Die Functionen des Centralnervensystems und ihre Phylogenese," 2te Abtheilung, Die Fische, 1888, s. 35.

[2] Sherrington in Schaefer's "Text-book of Physiology," p. 816. Loeb, *Arch. f. d. ges. Physiologie*, Bd. 96, s. 536, 1903.

[3] Grünbaum and Sherrington, *Proc. of Roy. Soc.*, Vol. 69, 1901.

[4] Loeb, "Comparative Physiology of the Brain and Comparative Psychology," 1901.

[5] Friedländer, B., "Physiologie des Centralnervensystem," *Arch. f. ges. Physiologie*, Bd. 58, s. 168, 1894.

muscles in earthworms was not disturbed by removal of a small piece of the nerve-cord, that the circular muscles failed to contract only in the region where the cord was removed and that elsewhere they continued to contract rythmically. If the posterior part of such a worm lay on a smooth surface it would allow itself to be dragged along by the part anterior to the section. If, however, the posterior part be dragged over a rough surface, moistened blotting paper for instance, its segments would begin active, rythmical contractions and coöperate with the anterior part in progressive locomotion.

That one part of the body in these worms was not dependent on the nerve-cord of the other part was still better shown in a worm which was cut cross-wise into two halves. The halves were fastened together again by a string. The anterior half, pulling on the posterior half, especially when the latter lay on a rough surface, would produce coördinated movements in the posterior half.

In another experiment only the posterior half was used. It was placed on papers of two grades of roughness, the anterior part on the rougher, the posterior part on the smoother paper. A pull on the rougher paper started up locomotor contractions in the foremost segments; the other segments remained inactive while being dragged over the smooth paper, notwithstanding the fact that the cord was intact in the entire piece of the animal which was under observation.

These phenomena in worms with severed nerve-cords were explained as follows: One segment, shortening, produces a pull on the next segment in the body and thereby stretches the skin of that segment. This stretching process sets up sensory impulses which, passing into their segmental ganglion, are reflected back as motor impulses to muscles of the same segment. The muscle contraction so produced in turn exerts a pull on the skin of the next segment and thereby gives rise to another cycle of sensory-motor impulses, which, as mere nerve-impulses, do not necessarily pass beyond the boundaries of the segment where they originated. And so on, from segment to segment, a motor impulse started at *a* may be transmitted to segments *b*, *c*, *d*, etc., without any impulses whatsoever passing over the fibers in the

nerve-cord which directly connect the ganglia. In short, coördinated locomotion is secured without the nerve cord, as such, coming into play at all. In their passage the nervous impulses involved, according to this conception, do not follow a straight path with side-paths at each segment, but rather follow a path which may best be described as a line of loops.

Friedländer concluded that locomotor movements in the earthworm should not be regarded as the result of a single impulse (say of the anterior segment) which passes through the length of the body, but rather should it be regarded as the result of impulses given off by each segment separately.

2. That coördinated movements in animals higher than worms may be set up and maintained, with the spinal cord brought into use only in spots, as it were, rather than as an entire and single organ, has been recently demonstrated in a remarkable way by the experiments of Phillipson.[1]

It has been known for some time[2] that, if a dog with a sectioned cord be supported so that its hind legs hang freely in space, the legs spontaneously begin an alternate flexor-extensor movement.

Phillipson obtained this so-called pendulum movement of the legs in a dog with sectioned nerve-cord. The movement lasted sometimes for more than an hour; it could be accelerated into a veritable gallop by gently pulling the tail of the animal. The valuable part of this experimenter's work, however, lies in the complete analysis which he was able to make of the various steps involved in the process of locomotion. From his experiments and observations on this dog with the severed nerve-cord, he drew conclusions concerning locomotion in the hind legs of normal dogs somewhat as follows:

The hind-foot of a dog during extension gently comes in contact with the ground; this foot-contact produces a feeble excitation which brings on a flexing of the metatarsals with the sole of the foot supported against the ground. The resistance of the ground against this flexing of the metatarsals produces a sensory-motor wave which brings a sudden halt to all muscular

[1] Phillipson, Maurice, *Comptes Rendus*, Vol. 136, p. 61, 1903.

[2] Freusberg, *Archiv f. die ges. Physiologie*, "Reflex Bewegung beim Hunde.," 1874, Bd. IX., s. 358.

action which in turn throws the body forward. The shock produced by the sudden halt of muscular action causes flexion of the limb which has just reached the limit of extension, and the pulling of the skin at the inguinal region by this same extension, starts up extensor movements in the opposite limb, which in its turn coming in contact with the ground goes through the same cycle of movements just completed in its fellow. And so on, the action of one leg determines that of the other, so that not only walking but running movements in the hind-legs may be, and probably are, kept up indefinitely, involving the use of no more of the nerve-cord than that part in which these simple sensory-motor impulses may be exchanged.

According to Phillipson's analysis it appears quite possible that, *after progressive locomotion has once been started in a normal dog, it might walk or even run a distance without any additional impulses passing down from the upper region of the spinal cord.*

3. The question now arises, *could normal walking movements in the hind legs of an animal be initiated without any impulses whatsoever, for the purpose, coming into the lumbar centers from levels of the cord higher, say, than the mid-dorsal region, or from levels higher than the lumbar region itself.*

Loeb[1] has already suggested that the pendulum movements in the hind-legs of a dog with severed nerve-cord may be caused by the stretching of the skin on the sides of the body produced by holding up the front part of the animal. He further held the opinion that if the beast could stand at all on its legs it probably could go through the coördinate movements necessary for walking. For, if the fore-legs took forward steps that action would produce a pull on the sides of the body, stretching the skin. This stretching would create sensory stimuli that would pass into the lumbar region of the cord whence extensor motor stimuli would pass into one leg or the other and thus start up the cycle of ambulatory motions described by Phillipson. This view of the possibility of coördinating anterior and posterior locomotion in a vertebrate with severed nerve-cord receives strong support from the experiments and observations of Friedländer. But it may be

[1] Loeb, Jacques, "Beiträge zur Gehirnphysiologie der Würmer." *Arch. f. d. ges. Physiologie*, 1894, 56, s. 268.

objected that results obtained from an animal of such pronounced metameric structure as that of the earthworm cannot be applied in the case of vertebrates; that if "the nerve-cord of the earthworm is an apparatus by means of which reflexes may be transferred from one segment to the other"[1] it does not follow that the nerve-cord of a vertebrate in its activity can be reduced to elements equally simple.

In uninjured land animals of the bilateral types progressive locomotion is usually initiated by forward or lateral movements of the anterior part of the body. This action produces an effect on the passive posterior part of the body, especially in the skin, where sensory stimuli may be set up. These sensory stimuli may be aroused in two ways. First, by tugging, the skin is subjected to stretching stimuli; second, by dragging even the slightest distance, the ventral surface of the body, and therefore of the skin of creeping animals, is subjected to a series of rapid, successive impacts against the inequalities of the ground-surface. In other words, by dragging *the skin is subjected to friction stimuli in addition to stretching stimuli.*

Now since the skin is always subjected to more or less such treatment in the initiation of locomotor movements, it is reasonable to suppose that a characteristic combination of sensory stimuli is thereby produced which may be able to set up motor stimuli in centers of corresponding levels. One may suppose moreover that these motor stimuli do not result in vague, purposeless reflexes, but rather that they result in definite, unvarying coördinations; that they are operative and effective without intervening stimuli from other regions of the nerve-cord.

In other words, *a definite combination of sensory stimuli in one region, occasioned by a definite motor activity in another region, may be a sufficient signal for locomotor activity in the region receiving such sensory stimuli.*

4. At the suggestion of Dr. Loeb, for whose constant assistance and kindly interest in my work I wish here to express my best thanks, I began some experiments during February of this year to test the truth of the ideas set forth in the foregoing paragraphs.

[1] Freidländer; *loc. cit.*, p. 206.

The animal selected for experimentation was *Batrachoseps*. This is a small batrachian with a long, slender body and ridiculously short, slender legs which, while perfectly functional, are not depended upon to carry on all the work of locomotion. The complete musculature of the body-walls and of the long, slender tail is evidence that they, too, at times assist mightily in locomotion. Indeed in the normal animal one sees that for leisurely movements only the legs are used, the body, always moist and somewhat slimy, being dragged passively along; and that for locomotion under pressure of unusual excitement the powerful body and tail muscles are brought into play. The body throughout its length is well marked off externally into segments, or metameres. One could not find an animal that combines the locomotor structures of both annelid and vertebrate better than does *Batrachoseps*.

There are twenty distinct segments between the girdles of these animals. Of the twenty-two specimens operated upon, the cord was severed between the fifth and sixth segments of some; between the tenth and eleventh of others; and of still others, between the fifteenth and sixteenth segments. In a few cases the section was made nearer the girdles, which always resulted in paralysis of the legs.

They usually recovered from the effects of the chloroform-ether mixture within five to fifteen minutes after taking it. The operation which consisted of simply snipping the spinal column in two with strong fine-pointed scissors, or destroying the nerve-cord by probing with a needle point, took only a minute or a minute and a half. Care was taken to injure as few of the muscles of the body-wall as possible and to avoid puncturing the peritoneum and the large blood-vessels, excepting in those cases where the animals were cut entirely in two.

In two specimens a piece of the spinal column about two mm. in length was removed. Shock effects occurred in only a few cases, which will be spoken of later on.

In all cases where the operation itself was successful and where the cord was not severed too near one or the other of the girdles coördinated movements were observed, not only between the members of the fore-legs and of the hind-legs, but also between the pairs themselves.

The animals operated upon were kept in broad flat-bottomed dishes. The floors of the dishes were kept covered with moistened layers of filter paper. During resting periods the animals were protected from the light of the room by being covered with fresh leaves or moist filter paper, and by covering over the dishes with towels.

When these coverings were removed the animals, usually asleep at the time, would sooner or later become aware that they were out in the open. They would then become restless. In their efforts to get under cover again one could note very easily the manner of their progress. Movements were usually initiated by the anterior parts turning to the one side and then to the other several times and finally back to the tail, often moving across it and twisting the body into a loop. During these movements the posterior parts would lie motionless and apparently helpless. After a number of these attempts at progressive locomotion the animal would start out straight ahead. The hind parts being unresponsive, it would tug and pull with the fore-legs in an effort to drag the helpless part along, and, in spite of the great length of the body and tail, and the extreme delicateness of the legs, the animal often succeeding in doing this.

The results of this tugging and dragging of the hind parts, while always the same, may be obtained by various methods, and were observed in my experiments as follows:

(*a*) Sometimes when the animals struck out in a forward direction and began to tug at the hind parts the hind-legs would begin to assist *before they were dragged in the least.* Their movements while slower than the fore-legs *coöperated toward forward locomotion*, and, furthermore, *were flexed and extended alternately*, that is, *they took forward steps alternately.*

(*b*) At other times no movements occurred in the hind-legs until the hind parts had been dragged along for a distance. The legs would then begin coördinate movements as described above. Sometimes the legs happened to be extended back along the sides of the tail and held up (surface tension of water and mucus alone being sufficient to support the weight of the legs) so that the toes did not touch the ground-surfaces. In such cases it was noticed that the hind-legs remained irresponsive for a longer time

than if they had been pendent enough so that the toes also touched and dragged along the ground.

(*c*) Coöperation on the part of the hind-legs could be started by touching with a pencil-point the skin of the sides of the body posterior to the section in the cord, or by touching the toes or the heels.

(*d*) A number of specimens would walk perfectly normally on their hind-legs when the front part of the body was held up and carried forward slowly. The walking movements would begin sometimes at once without dragging, sometimes after the hind-legs were dragged for a distance.

Experiments were made cutting *Batrachoseps* entirely in two as Friedländer had done with earthworms. The section was made at various levels between the girdles. Nothing but simple reflexes were obtained from the posterior halves although the pieces lived for days. The anterior halves, however, seemed to suffer no depression whatever. As soon as they recovered from the effects of the anæsthetic, they moved about in a manner that, with the exception of the awkwardness produced by the absence of the customary weight and resistance of the postetior parts and of the exhaltation produced by the operation, was perfectly normal. One of these anterior pieces lived for twenty-four hours. An hour before it died it was still breathing and moving about normally.

These results are about what one would expect to find in *Batrachoseps*. For, unlike earthworms, they cannot depend alone on the skin for respiration or upon an uncertain circulation. After sectioning the body the posterior half is deprived entirely of the benefits of the pharyngeal breathing and of the heart-beat, which continue on in the anterior half uninterrupted. After cutting an earthworm in two, respiration and the circulation can go on in the posterior half as well as they do in the anterior half. Hence the nervous responses in the one half are not different from what they are in the other half.[1]

5. *Summary and Conclusions.* — The results of the foregoing experiments on *Batrachoseps* may be summed up, and conclusions may be drawn, as follows :

[1] Friedländer, *loc. cit.*, p. 190.

(*a*) The coördinating center of locomotion in the hind-legs is located between the sixteenth and the eighteenth segments of the trunk.

(*b*) Walking movements in the hind legs after the spinal-cord is severed are due primarily not to impulses arising in the spinal centers, but to impulses that arise in the sensory cells of the periphery.

(*c*) These sensory impulses may be produced in the skin and muscles of the body-walls by stretching-stimuli; in the skin of the body-walls and of the legs by mechanical-impact-stimuli (friction- or tapping-stimuli).

(*d*) The fact that *Batrachoseps* with sectioned cords walk on their hind-legs when carried along by their fore-legs supports Loeb's idea that the same thing would obtain in dogs with sectioned cords if they could only stand up, or if their legs were shorter so that they might be effective in their movements when the trunk is prostrate. The difficulty in the dog is not due to the absence of the walking apparatus, but to the absence of "tone" in the muscles.

(*e*) Perhaps the essential thing supplied by the nerve-cord, as such, to the limbs during locomotion is this tone, this complimentary resistance of the muscles toward each other which keeps them ready for instantaneous regulated action, and which is probably maintained by impulses passing down from the organs or centers of equilibrium.

(*f*) If respiration could be properly maintained in the posterior half of *Batrachoseps* after being severed entirely from the anterior half, it is probable that walking movements could be started in the hind-legs by stretching-, or friction-, or tapping-stimuli, that is, by tugging gently at the front end of the posterior half, or by dragging it a distance over moist blotting paper, or by fastening it by means of a string to the anterior half and allowing the latter (which can walk along spontaneously) to pull at, or drag, the former along.

The R. Spreckels Physiological Laboratory,
Berkeley, California.

BUTLER'S GARTER SNAKE.

ALEXANDER G. RUTHVEN.

[From the University Museum, University of Michigan.]

The garter snake, *Eutænia butleri* Cope, has been the subject of considerable discussion among herpetologists in spite of the fact that only five specimens were known. In 1888, Professor Cope ('89, p. 399) first recognized and described the species on the basis of a single specimen (Purdue Univ. Catalogue, No. 264) sent to him by Mr. A. W. Butler. This specimen was thought to have come from Richmond, Indiana, but it was unlabeled when Mr. Butler received it from Purdue University, so it may or may not have come from there. In 1895, Mr. Reddick ('96, p. 261) took another specimen (Univ. of Ind. Cat. No. 110) at the Indiana University Biological Station at Turkey Lake, Kosciusko County, Indiana. There are two specimens in the museum of the Academy of Natural Sciences of Philadelphia (Cat. No. 6523) labeled by Professor Cope, which are described by Mr. A. E. Brown ('01, p. 27) and credited by him to southeastern Indiana. But in a letter April 15, 1904, he says concerning these specimens, "The locality given is Miami river, which probably means Ohio." The fifth specimen recorded (U. S. N. M. Cat. No. 21692) was collected by Mr. Philip Kirsch at Waterloo, Indiana, and is described by Dr. Stejneger ('94, pp. 593–594).

Professor Cope examined the type and on the basis of its distinctive characters considered it a good species, a view supported by Dr. Stejneger after a study of the specimen from Waterloo, Indiana. Mr. Brown, however, upon a study of the two specimens in the collection of the Philadelphia Academy of Natural Sciences, and for the reason that there were so few specimens known, considered them anomalies of *E. sirtalis sirtalis*, with considerable reason, for it seems as if four specimens (the specimen from Turkey Lake is described for the first time in this paper) is entirely too small a number upon which to form a species. Particularly is this true in a genus like *Eutænia*, in which

the individual variation is so great. It was, therefore, with considerable interest that I found last winter, while working over the collection of reptiles in the University Museum at the University of Michigan, a number of specimens of this rare snake. I have been able to make a study of the character of these specimens, and to supplement this by a study of the coloration and, to a certain extent, of the habits of a number of living specimens which have been collected about Ann Arbor. The present paper embodies the results of this investigation, and the data on all the other specimens recorded.

Through the courtesy of Dr. W. J. Moenkhaus, the specimen taken at Turkey Lake, Ind., has been examined, and Dr. Stejneger has very kindly sent me a detailed description of the specimen from Waterloo, Ind. I am indebted to Mr. A. E. Brown for a description of the specimens in the Museum of the Academy of Natural Sciences of Philadelphia, who also examined two specimens from Ann Arbor, Mich., and pronounced them the same form as those in his possession. I wish also at this time to acknowledge my indebtedness to Mr. Chas. C. Adams, Curator of the University Museum, University of Michigan, for assistance in the prosecution of this work and in the publication of the results.

I have been unable to examine the type, which according to Professor Cope is Cat. No. 264 at Purdue University. The specimen sent to me as the type is Cat. No. 81, and while labeled *E. butleri*, is not that form, but a normal specimen of *E. sirtalis sirtalis*, and corresponds in no way to Professor Cope's description of the type. I have also, through the kindness of Professor S. Coulter, examined the garter snakes in the Butler collection at Purdue University, but found no specimens of *butleri* among them.

It is important to diagnose this form, as it is very probable that many specimens are at present stored in museums under other names or undetermined. In this connection, one calls to mind a paper by Dr. H. L. Clark of Olivet College, Olivet, Michigan, on the occurrence of the short mouthed snake in Michigan ('03, pp. 83–88). Last year Dr. Clark found six specimens of a snake, which upon the authority of Mr. S. Garmen and Dr. Stejneger,

he called *E. brachystoma* Cope. I have, through the courtesy of Dr. Clark, examined three of these specimens and we both agree that they are the same form as those which I identified as *E. butleri*. The only specimen of *E. brachystoma* ever recorded is in the collection of the Philadelphia Academy of Natural Sciences and Mr. Brown says of this specimen that, "There is little ground for thinking it other than a dwarfed *E. sirtalis sirtalis*" ('01, p. 28). After a comparison with specimens of *E. butleri* from here, Mr. Brown writes me as follows, "I have reëxamined the type of *E. brachystoma* Cope, and still find no reason to believe it other than an anomalous *E. sirtalis sirtalis*. Cope says 'it is small but not young,' I myself see no way to determine this, it may or may not be so. The specimen was about ready to shed when he got it, which of course obscured the pattern. I cannot think it is the same as *E. butleri*."

Dr. Stejneger suggested ('94, p. 594) that the specimen collected near Chicago in 1874, by Mr. E. W. Nelson, and labeled by Davis and Rice *E. vagrans* ('83, p. 30), might prove to be another specimen of *butleri*, but through the courtesy of Dr. U. S. Grant, Curator of the Museum at Northwestern University, I have been able to examine this specimen, and it is undoubtedly an *E. vagrans*. It differs from *butleri* most noticeably in having the dorsal scales in 21 rows, 8 supralabials on the right side and 9 on the left, and in the larger number of urosteges and gastrosteges, which are respectively 86 and 170.

Habits.

Very little has been recorded on the habits of this snake. Its movements as Mr. Reddick describes them, are extremely awkward. It seems compelled to exert much effort in crawling, and its efforts to escape capture are often ludicrous. In captivity it takes readily to water, and is an exceedingly graceful swimmer. Its awkwardness in crawling, contrasted with its ease of motion in water, together with the fact that all the specimens recorded have been taken near water, bears out the statement of Dr. Clark ('03, p. 87) that "Although not aquatic this species likes water and is found only in its immediate vicinity." It is one of the first Michigan snakes to come out in the spring; a

single specimen having been taken as early as March 18, which is the earliest date recorded for any snake from this locality. The latest date at which a specimen has been taken is September 22, but the time of hibernation is undoubtedly much later than this. It is an exceedingly amiable little snake, and no amount of rough handling will induce it to strike, which is in marked contrast to the pugnacity of *E. sirtalis sirtalis*, when captured. As far as I have been able to observe, its diet in captivity consists chiefly of earthworms and small frogs. Its breeding habits are unknown; in one specimen (U. of M. Mus. Cat., No. 31612) taken May 28, 1904, at Sandusky, Ohio, there are 8 eggs in each oviduct about 20 mm. long and 12 mm. broad. In another specimen (U. of M. Mus. Cat., No. 31624) taken June 3, 1904, at Ann Arbor, Michigan, there are 7 eggs in the right and 5 in the left oviduct. These eggs are about 12 mm. long and 10 mm. broad.

Distribution.

The geographical range of *butleri*, as we know it, is very limited. As will be seen from the table, it occurs in the northern and, if the locality of the type is correct, the southeastern parts of Indiana, in the north and southwestern parts of Ohio and in southern Michigan. It apparently cannot be abundant in Indiana or more specimens would have been taken, as many reptiles have been collected there and the state has been worked by Dr. O. P. Hay, who describes ('92, p. 528) the type as being the only specimen known at that time. In southern Michigan, however, it occurs quite commonly, at least near Ann Arbor. Of its occurrence in other parts of Michigan, nothing can be said, except of the six specimens taken by Dr. Clark at Olivet.

Diagnosis.

Body stout, tapering abruptly at both ends. Head conical, smaller than neck, muzzle a little protuberant. Tail short. Dorsal scales in 19 rows, all carinated, the first much the largest. Supralabials 6–6, the fifth the largest, the third and fourth, entering the orbit. Infralabials 8–8. Preoculars 1–1. Postoculars 3–3. Temporals 1–1, the second large, extending from labials to parietals. Gastrosteges 140. Urosteges 61. Length

230 mm. Tail 60 mm. Color above olive brown, with two alternate rows of black spots above the lateral stripe, and one below; the dorsal spots forming a broken border to the dorsal stripe. Color below light olivaceous, sparsely speckled with black, with a row of gastrostegeal spots on either side. The dorsal stripe occupies the median and the two half rows of scales on either side; anteriorly bright yellow, it extends well up between the parietals; posteriorly it fades out quickly to a greenish yellow, and loses its distinctness on the tail. Color below the lateral stripe the same as above, olive brown. Lateral stripes anteriorly bright sulphur yellow, broad, occupying all of the third, the upper half of the second and the lower half of the fourth rows of dorsal scales on the anterior half of the body. On the posterior half they occupy exclusively the second and third rows to the vent; but they never descend below the middle of the second row; at the vent they widen out to cover part of the first, all of the second and third, and all of the fourth rows; posteriorly to the vent, olive in color, occupying but one row of scales, the first, and becoming more and more indistinct toward the end of the tail.



FIG. 1. Eutænia butleri Cope. Ann Arbor, Washtenaw Co., Michigan, Cat. No. 30460, U. of M. Museum.

Anteriorly the lateral stripe expands to form a large yellow patch on either side of the neck, in which occur two black spots one on the right side, and one on the left. These spots are continuations of the row below the lateral stripe. There is also a black spot, just above the sixth supralabial, that is prolonged in front to the lower edge of the upper postocular. Above the black line formed by the continuation of this spot, the head is uniformly brownish olive, with the exception of a black patch on the parietals in which are located the prominent bright yellow parietal spots. The supralabials are dusky yellow, narrowly margined with black; the yellow extending up to the black line, thus including the two lower postoculars, and part of the first temporal. In front of the eye, the yellow of the supralabials extends onto the preocular, where it gradually fades into the olive-brown of the top of the head. The eye is surrounded by a nar-

row ring of black; as is also the nostril. There is also a black line between the preocular and loreal. Chin white. Throat yellowish.

The specimen upon which I have based the above description as being typical of the form, since I have been unable to examine the type, is a specimen taken on the bank of the Huron river, near Ann Arbor, Michigan, by myself, September 22, 1903 (U. of M. Mus. Cat. No. 30642). It is not a mature specimen, and for this reason the color pattern is more distinct than in older specimens, for the ground color tends to become darker and to obscure the spots in adults. This is the only difference except in size to be observed between old and young specimens.

Variation.

Contrary to the usual tendency among the species of *Eutænia*, the specimens of *butleri*, which I have examined, show but little individual variation from the type as described by Cope, or the specimen which I have chosen as being most nearly normal. It is an interesting coincidence, that all the specimens previously recorded (Phila. Acad. of Sci. Cat. No. 6523, U. S. N. M. Cat. No. 21692, and Purdue Univ. Cat. No. (type) 264), possess 7 supralabials on either side, while in the specimens which I have examined these are nearly always 6–6, but occasionally 6–7 and in three cases 7–7; when there are 6 supralabials, the fifth is always the largest; when there are 7, the extra one is apparently formed by a divison of the fifth, as may be seen in two cases (U. of M. Mus. Cat. No. 30683 and 30506), in which the fifth is only partially divided. The supralabials are nearly always 8–8; in two cases (U. of M. Mus. Cat. No. 30471–30407*a*) they are 9–9, and in one case the extra one is on one side only. The oculars are normally 1–3, with an occasional variation to 1–2. Concerning the disputed second temporal, there is in most cases a single large temporal in the second row, as is given by Prof. Cope for the type. When there are two, the upper is always very much the smaller, and generally so much so as to be surrounded by the lower on the lower and posterior sides. In specimen No. 6523b Phila. Acad. of Sciences, the lower one of the second row seems to have divided length-

wise so as to form a long narrow scale which is in contact with the first temporal. The gastrosteges vary in number in 20 specimens from 131–154 with an average of 139; the urosteges in 19 specimens from 50–71 with an average of 60. The coloration is remarkably constant, the only differences observed being the presence of lighter ground color in the young which causes the lateral spots to stand out more prominently than in the older specimens, where the color varies to a brownish black, which may quite obscure the spots.

For convenience, the data on the specimens previously described and on fifteen specimens which I have examined have been tabulated on following pages. It will be seen from this table that there is, as was shown above, some individual variation, yet the distinctive characters are so pronounced and constant, that in the case of none of the thirty specimens examined was I in doubt as to where to place a single individual.

Affinities of Butleri.

Professor Cope's statement, that *E. butleri* "is remarkably distinct from anything which occurs in the United States" is born out by the specimens now at hand. As was mentioned above, the range of variation is never great enough to make the determination of a specimen doubtful. They resemble in this region the *E. sirtalis* group more closely than any other, and it was for this reason that Mr. Brown considered his specimens aberrant forms of *sirtalis sirtalis*. The finding of so many specimens, however, makes this view untenable, and the question then arises, whether it is to be considered as a variety of *E. sirtalis*, or as a distinct species.

The number of gastrosteges, and superior and inferior labials are normally less than in any *E. sirtalis* except *E. s. leptocephala*. The gastrosteges in one instance are 154, a common number in *sirtalis sirtalis*, and the supralabials are sometimes seven, the normal number for *sirtalis sirtalis* which also occasionally has nine infralabials, as occurs in two specimens of *butleri*. It might seem from this, as Mr. Brown has suggested to me, that *butleri* bears the same relation to *E. s. sirtalis* as *E. s. leptocephala* does. He regards *E. s. leptocephala* as a degenerate form of *E. s. sirtalis*,

Specimens.	Locality and Date.	Supra-labials.	Infra-labials.	Oculars.	Tem porals.	Uro-steges.	Gastro-steges	Total Length.	Length of Tail	Collector.
1. Purdue Univ., Cat. No. 264. (Type.)	Richmond, Wayne Co., Indiana. (?)	7	8	1–3	1–1	62	144			
2. U. S. N. M., Cat. No. 21692.	Waterloo, Steuben Co., Indiana, July 17, 1893.	R 7 L 7	R 8 L 9	R 1–3 L 1–2	R 1–2 L 1–2	57	131	530mm	115mm	Philip Kirsch.
3. Univ. of Ind., Cat. No. 110.	Turkey Lake, Kosciusko Co., Indiana.	R 6 L 7	R 8 L 8	R 1–3 L 1–3	R 1–2 L 1–1	60	134			G. Reddick.
4. Philadelphia Acad. of Nat. Sci., Cat. No. 6523*a*.	Miami River, Ohio. (?)	R 7 L 7	R 8 L 8	R 1–3 L 1–3	R 1–2 L 1–2	71	144	475mm	107mm	
5. Philadelphia Acad. of Nat. Sci., Cat. No. 6523*b*.	Miami River, Ohio. (?)	R 7 L 7	R 8 L 8	R 1–3 L 1–3	R 1–2 L 1–2	68	142	440mm	112mm	
6. Univ. of Mich., Cat. No. 30642.	Ann Arbor, Washtenaw Co , Mich., Sept. 22, 1903.	R 6 L 6	R 8 L 8	R 1–3 L 1–3	R 1–1 L 1–1	61	140	230mm	60mm	Riley & Ruthven.
7. Univ. of Mich., Cat. No. 30641.	Ann Arbor, Washtenaw Co., Mich., Sept. 22, 1903.	R 6 L 6	R 8 L 8	R 1–3 L 1–3	R 1–1 L 1–1	57	136	220mm	50mm	Riley & Ruthven.
8. Univ. of Mich., Cat. No. 30863.	Chelsea, Washtenaw Co., Mich., Oct 28, 1903.	R 7 L 7	R 8 L 8	R 1–3 L 1–3	? ?	62	138	240mm	55mm	E. N. Transeau.
9. Univ. of Mich., Cat. No. 30460.	Ann Arbor, Washtenaw Co., Mich., April 28, 1903.	R 6 L 6	R 8 L 8	R 1–2 L 1–3	R 1–1 L 1–1	50	154	415mm	90mm	N. A. Wood.
10. Univ. of Mich., Cat. No. 30506.	Chelsea, Washtenaw Co., Mich., May 10, 1903.	R 6 L 6	R 8 L 8	R 1–3 L 1–3	R 1–2 L 1–2	58	135	450mm	110mm	Chas. C. Adams.
11. Univ. of Mich., Cat. No. 30109.	Ann Arbor, Washtenaw Co., Mich., March 18, 1903.	R 6 L 6	R 8 L 8	R 1–2 L 1–2	R 1–1 L 1–1	54	140	480mm	105mm	N. A. Wood.
12. Univ. of Mich., Cat. No. 30471.	Ann Arbor, Washtenaw Co., Mich., May 2, 1903.	R 6 L 6	R 9 L 9	R 1–3 L 1–3	R 1–1 L 1–2	55	140	415mm	90mm	Chas. C. Adams.
13. Univ. of Mich., Cat. No. 30407*a*.	Ann Arbor, Washtenaw Co., Mich., April 7, 1900.	R 6 L 6	R 9 L 9	R 1–3 L 1–3	R 1–1 L 1–1	Tail broken	137			Ida M. Hopson.

Specimens.	Locality and Date.	Supra-labials.	Infra-labials.	Oculars.	Tem-porals.	Uro-steges.	Gastro-steges.	Total Length.	Length of Tail.	Collector.
14. Univ. of Mich., Cat. No. 30407*b*.	Ann Arbor, Washtenaw Co., Mich., April 7, 1900.	R 6 L 6	R 8 L 8	R 1–3 L 1–3	R 1–2 L 1–2	67	139	406mm	106mm	Ida M. Hopson.
15. Univ. of Mich., Cat. No. 31383.	Ann Arbor, Washtenaw Co., Mich., April 27, 1901.	R 6 L 7	R 8 L 8	R 1–2 L 1–2	R 1–1 L 1–2	61	141			Florence J. Hagle.
16. Univ. of Mich , Cat. No. 31613.	Sandusky, Erie Co., Ohio, May 28, 1904.	R 7 L 6	R 8 L 8	R 1–3 L 1–3	R 1–2 L 1–2	62	138	445mm	105mm	E. L. Moseley.
17. Univ. of Mich., Cat. No. 31614.	Sandusky, Erie Co., Ohio, May 28, 1904.	R 6 L 6	R 8 L 8	R 1–3 L 1–3	R 1–2 L 1–2	60	136	465mm	112mm	E. L. Moseley.
18. Univ. of Mich., Cat. No. 31612.	Sandusky, Erie Co., Ohio, May 28, 1904.	R 7 L 7	R 8 L 8	R 1–3 L 1–3	R 1–1 L 1–1	60	139			E. L. Moseley.
19. Univ. of Mich., Cat. No. 31627.	Brighton, Livingston Co., Mich., April 23, 1904.	R 7 L 6	R 8 L 8	R 1–3 L 1–3	R 1–2 L 1–2	63	146			B. K. Burgess.
20. Univ. of Mich., Cat. No. 31628.	Brighton, Livingston Co., Mich., April 23, 1904.	R 7 L 7	R 8 L 8	R 1–3 L 1–4	R 1–2 L 1–2	55	139			B. K. Burgess.

a view which is also held by Professor Cope ('92, p. 660) and Dr. Stejneger ('93, p. 214). This view is perhaps supported by the great variability of *leptocephala*, but *leptocephala* occurs in a distinct region while *butleri* occurs in the same region, in the same habitat and in almost equal abundance with typical forms of *E. s. sirtalis;* and at the same time its distinctive characters are so constant, that no variations have been found that can be regarded as transitional forms between the two species. The average number of gastrosteges in *butleri* is about 139 while in *sirtalis sirtalis* it is about 151. The temporals are also more constant than appears in the table; it is true that there is not always a single large temporal in the second row, but when there are two as in *sirtalis sirtalis*, the upper one, as far as we know is always much smaller than the lower one, which lies posterior and inferior to it. The form and coloration are, however, the most characteristic features. The body is more robust, the tail shorter, and the head smaller and more conical than in *sirtalis sirtalis*. As stated under variation, the color is remarkably constant for a member of this genus and while there is much color variation in the *E. sirtalis* group, I have never seen a variation that could be confused with *butleri*. Its striking coloration, robust form, small indistinct head and peculiar movements, make this snake very conspicuous and renders it almost impossible to confuse it with any other form. I have not seen enough specimens to determine its relationships with other groups, but the coloration, position of the lateral stripe, and reduced number of infralabials point strongly toward *E. radix* which meets it on the west.

Desirable as it is, therefore, to cut down the species in this genus, it must, I think, be concluded, from the specimens which have been collected that *Eutænia butleri* is a good species.

SYNONYMY.

1889. *Eutænia butlerii*, Cope, Proc. U. S. Nat. Mus., XI., p. 399.

1892. *Eutænia butlerii*, Cope, Proc. U. S. Nat. Mus., XIV., p. 651.

1892. *Eutænia butlerii*, Hay, 17th Annual Report of the Ind. Dept. of Geol. and Natural Resources, p. 120.

1893. *Tropidonotus ordinatus*, var. *butleri*, Boulenger, Cat. Snakes Brit. Mus., I., p. 212.

1894. *Thamnophis butleri*, Stejneger, Proc. U. S. Nat. Mus., XVII., p. 593.

1903. *Eutænia brachystoma*, Clark, Proc. Biol. Soc. of Wash., XVI., p. 83.

REFERENCES.

Brown, A. E.

'01 A Review of the Genera and Species of American Snakes, North of Mexico. Proc. of the Acad. of Nat. Sciences of Phila., January, 1901, pp. 10–110.

Clark, H. L.

'03 The Short Mouthed Snake (*Eutainia Brachystoma* Cope) in Southern Michigan. Proc. Biol. Soc. of Wash., Vol. XVI., pp. 83–87.

Cope, E. D.

'89 On the Eutænia of Southeastern Indiana. Proc. U. S. Nat. Mus., Vol. XI., p. 399.

'00 Crocodilians, Lizards, and Snakes of North America. Annual Report U. S. Nat. Mus., 1898, pp. 153–1270.

'92 A Critical Review of the Characters and Variations of the Snakes of North America. Proc. U. S. Nat. Mus., Vol. XIV., pp. 589–694.

Davis and Rice.

'83 List of Batrachia and Reptilia of Illinois. Bulletin of the Chicago Academy of Sciences, I., pp. 25–32.

Hay, O. P.

'92 Batrachians and Reptiles of the State of Indiana. 17th Annual Rept. of the Ind. Dept. of Geology and Nat. Resources, pp. 409–602.

Reddick, G.

'96 Snakes of Turkey Lake. Proceedings of the Ind. Acad. of Science 1895. pp. 261–262.

Stejneger, L.

'94 Notes on Butler's Garter Snake. Proc. U. S. Nat. Mus., Vol. XVII., pp. 593–594.

'93 Annotated List of the Reptiles and Batrachians Collected by the Death Valley Expedition in 1891, with Descriptions of New Species. North American Fauna, No. 7, Part II., pp. 159–228.

TENACITY OF LIFE IN ANTS.

ADELE M. FIELDE.

Maimed Ants.

Remarkable tenacity of life is sometimes exhibited by ants lacking a portion of the body.

A queen, *Stenamma fulvum piceum*, deprived of the funicles of her antennæ, lived fourteen months in one of my artificial nests, where she laid eggs, sought her own food, and received kindly treatment from the resident workers and several unmutilated queens.

The head of a *Formica fusca subsericea* worker under my observation, continued to move its antennæ seven hours after decapitation.

Ants lacking a leg or two may live several weeks. A *Stenamma fulvum* worker, deprived of its mesothoracic pair of legs, was returned by me to an artificial nest where were hundreds of its former comrades and it safely lived there a month or more, disproving any general assertion that ants destroy maimed members of their colony.

Worker ants deprived of the abdomen sometimes run with great speed, continue to care for the young in the nest, fight with aliens of their own or other species, and they may for some days behave as if unconscious of loss. A *Stenamma fulvum* queen deprived of her abdomen lived thereafter for fourteen days in one of my artificial nests, and was seen to eat. A *Formica subsericea* worker lived without her abdomen for five days.

M. Charles Janet mentions[1] an ant that lived nineteen days after decapitation. In experiments made by me, headless ants have continued to walk about for many days. In all these experiments aseptic surgery was attempted, the instruments used being carefully sterilized. The Petri cells in which the maimed ants were

[1] "*Extrait des Compte réndus hebdomadaires des Séance de l' Académie des Sciences*," Paris, 11 juillet, 1898, p. 130.

kept were frequently cleansed with 80 per cent. alcohol, and only distilled water was used in wetting the enclosed sponges. Care was taken to maintain hygienic conditions at all times during the life of the ant. In these experiments a *Stenamma fulvum* worker lived ten days without her head. A *Formica subsericea* worker lived fifteen days without her head. Of seven decapitated *Camponotus pennsylvanicus* workers, three lived five days; two lived twenty-one days; one lived thirty days; and one lived forty-one days. The last two mentioned were the largest, being thirteen millimeters long. After decapitation, they were kept for four days at a temperature of about 10° C. or 50° F. and afterward in the natural summer temperature of the laboratory.[1] The ant that lived forty-one days after decapitation walked about in the cell until two days before her death, and during the last two days gave evidence of life by a twitching of the legs when I touched her.

Submergence.

While making experiments in June, 1904, with a view to ascertaining how long ants could live under water,[2] I came to suspect that the death of ants submerged less than seventy-two hours was caused by bacteria rather than by deprivation of oxygen. Later in the summer I therefore made further experiments, merging the ants in distilled water, and keeping them in the dark at a temperature of about 10° C. or 50° F. Under these conditions the ants survived much longer periods of submergence.

Of eighteen *Stenamma fulvum* submerged four days, seventeen revived and twelve fully recovered.

Of fourteen *Stenamma fulvum* submerged six days, six revived, and one fully recovered.

Of twelve *Stenamma fulvum* submerged eight days in sixty-five cubic centimeters of water, seven revived and fully recovered.

Of seven *Camponotus pennsylvanicus* submerged eight days, four revived, fully recovered, and were returned to their old associates.

[1] Nearly all the experiments recorded in this paper were made at the Marine Biological Laboratory at Woods Holl, Mass., during the summer and autumn of 1904.

[2] "Observations on Ants in their Relation to Temperature and to Submergence," A. M. Fielde, Biological Bulletin, Vol. VII., No. 3, August, 1904, p. 170.

The ants recovering after submergence were those of large stature among their kind.

Dwarf Ants.

The ability of the larva to successfully enter the pupal-stage of development at any time after about half its normal size has been attained, helps to assure the persistence of a harried community. Under propitious conditions the larva grows to the size of an adult ant, expels the contents of the alimentary canal, and eats nothing during the five or ten days preceding pupation. But if suddenly deprived of food it may enter the resting stage when half-grown and may ultimately become a perfectly formed dwarf. I sequestered many fat, healthy, half-grown larvæ of *Stenamma fulvum*, put them in the care of fewer nurses than could regurgitate food to all of them, and supplied but little nutriment to the segregated group. Many of the larvæ soon entered the resting stage and later on became dwarf workers, only three or four millimeters in length, while the length of workers of their species is usually from five or seven millimeters. A corresponding diminution in the stature of a man would take from one to two feet from his height. The dwarf workers were wholly normal in their faculties and activities, and were markedly assiduous in their care of the young. On the other hand, larvæ poorly fed may miserably linger, as if waiting for better times. In one case under my observation, an ill fed larva remained such for one hundred and forty days, although constantly in summer temperature, and it then died without pupating.

Deprivation of Food.

Although ants manifestly suffer and soon die if deprived of water, they can exist for many days without food. In the following tests the ants were kept in Petri cells, ten centimeters in diameter, and not more than five ants were enclosed in any one cell. All the cells were kept in darkness or very dim light. At intervals, never exceeding four days, the cells and the enclosed sponges were cleansed with 80 per cent. alcohol, to prevent microscopic growths which might furnish nourishment to the ants. The cells contained only the ants used in the experiment, and a

bit of sponge saturated with distilled water. Care was taken to ensure good ventilation and all other conditions were made hygienic, that there might be no cause of death other than that of deprivation of food. The starving ants did not manifestly weaken after long abstinence but collapsed suddenly and finally, giving no premonitory evidences of exhaustion.

Of thirty *Cremastogaster lineolata*, segregated on July 2, 1904, ten lived so long as ten days and only one lived so long as eighteen days without food.

Of thirteen *Camponotus herculeanus pictus* workers, two lived seven days; two, fourteen days; one, eighteen days; one, twenty-three days; two, twenty-four days; one, twenty-six days; and one, twenty-nine days. On the tenth day of fasting, I found three of these ants killed and dismembered, with an appearance of having been chewed. In all the cells, this was the only group where the ants attacked their companions in tribulation. As ants sometimes quarrel with their mates when food is plentiful, this affray cannot be fairly attributed to a cannibalistic tendency in this species.

Of nine *Stenamma fulvum* workers, two lived eighteen days; one, twenty-seven days; one, twenty-nine days; one, thirty-two days; two, thirty-six days; one, thirty-eight days; and one, forty-six days. The last to die measured seven millimeters.

Of eight *Camponotus pennsylvanicus* workers, one lived fourteen days; two, eighteen days; one, twenty-one days; one, twenty-two days; one, thirty-nine days; one, forty-five days, and one, forty-seven days. The last two mentioned were fourteen millimeters long, and were larger than any of those that died earlier.

Of five *Formica lasiodes* workers, one lived ten days; two, eighteen days; one, thirty-nine days. An isolated queen of this species, lived from July 2 to August 31, just sixty days. During her isolation, she deposited seven eggs, the seventh being laid on the twenty-first day of her fasting. Any egg discovered in the cell was at once removed.

Of nine *Formica fusca subsericea* workers, picked up from a roadside and segregated without feeding on July 3, one lived ten days; one, seventy-one days; and the other seven were all alive on October 18. Possibly the remarkable capability of these ants

to live without food, enhances their value in slavery, where other species so often hold them.

Of two *Camponotus castaneus americanus* workers,[1] measuring thirteen millimeters, one lived fifty-four days, and the other lived more than a hundred days.

Two winged queens of *Camponotus americanus*, eighteen millimeters in length, and at least three months old, were established in a separate cell on July 13. One of them dropped her wings on July 31, while the other continued to wear her wings. No eggs were laid by either and both were alive on October 18.

Elimination of Inedible Substances.

The skill with which ants eliminate from their food supply such inedible substances as may be commingled therewith, was shown in the action of *Stenamma fulvum piceum* toward certain dye-stuffs that I mixed with their nutriment. Into each of four similar Fielde nests, C, I, T, and M, I put one queen and fifty workers, with a few half-grown larvæ. During three months no food was given to these ants other than what is hereinafter mentioned. The dye-stuffs were first triturated, molasses was added to make with them a thick paste, and a portion of the paste was then placed in the food-room of the nest. For the C nest, cochineal was commingled with the molasses; for the I nest, indigo; and for the T nest, tumeric; while for the M nest, molasses alone was provided. During the three months hardly any ants died in either nest. There was no evidence that any larva was devoured; and as the introduced larvæ appeared in due time in active life, they must have been nourished solely upon regurgitated food. In only one of the nests were any eggs seen, their absence probably being due to deprivation of insect food.

In all of the nests the finely pulverized inedible substances mixed with the molasses were separated in the mouths of the ants from the nutrient fluid, and were cast out in minute pellets, forming a characteristically colored heap in a corner of each nest, C, I, and T. In the M nest, a smaller pile of brown pellets indicated that non-nutritious particles had been rejected from the unmixed molasses.

[1] These ants were kindly identified for me by Dr. W. M. Wheeler.

Such preclusion of innutrient matter from the alimentary canal must greatly conserve the physical energy of the ants in the processes of digestion.

Avoidance of Poisons.

Ants that had fasted ten days did not partake of sweets in which poisons were incorporated, although their equally hungry mates ate the unpoisoned sweets with avidity.

When the ants were compelled to walk over a mixture of one gram of corrosive sublimate with two cubic centimeters of molasses, they afterward cleaned their feet with tongue and mandibles, and then evinced much distress in cleaning their mouths, but nearly all of them survived the experience.

When one gram of potassium cyanide was dissolved in five cubic centimeters of molasses, and the ants compelled to walk upon the solution, they appeared to die within a few seconds after touching the feet with the tongue, but they all revived some minutes or hours later and continued their normal activities.

When one gram of carbolic acid crystals was dissolved in two cubic centimeters of molasses, the ants compelled to walk upon the solution cleaned their feet with their tongues and mandibles, evinced much distress, and died after some hours or days, with no subsequent resuscitation.

In the experiments with poisons, the ants employed were *Cremastogoster lincolata*, *Stenamma fulvum*, *Lasius latipes*, *Formica subsericea*, *Camponotus pennsylvanicus* and *Camponotus castaneus americanus*. In these experiments the largest ants were latest in succumbing to the effects of the administered poisons. *Camponotus americanus*, about thirteen millimeters in length, lived several days after the administration of the carbolic acid, and in a natural environment might possibly have remedied their ills.

Regurgitation of Food.

Whether the regurgitation of food be a simple reflex or an altruistic act, it was practiced by some of the ants when there was little to confer upon a starving comrade. One *Camponotus pennsylvanicus* worker was seen to make unsuccessful effort to regurgitate food to another on the thirty-first, and also on the thirty-sixth day of fasting.



FIG. 2. *Camponotus castaneus americanus*, slightly magnified, showing five workers engaged in the regurgitation of food to their comrades, and four winged queens. From a photograph by Mr. J. G. Hubbard and Dr. O. S. Strong.

A winged queen of *Camponotus americanus* regurgitated food to her deälated sister queen, on the twenty-fifth, the thirty-fifth, the fortieth and the sixty-second day of fasting, the visible transfer of food occupying several minutes.

The ants cannot, however, be considered to virtually possess a common stomach. There was great difference in the periods within which ants of the same colony and species, in the same cell, perished from deprivation of food.

The feeding of the larva by different ant-nurses doubtless conduces to its vigor, because the nurses forage in diverse localities bringing back nutriment containing unlike chemical elements which they transfer by regurgitation to the growing young.

Doubtless the adult ants also benefit greatly through the habit of regurgitating food to each other. Going afield in many directions, one finds nectar, another berries, another nut-kernels, another insect flesh, another egg-yolk, and many give of their garnered nutriment to their companions in the nest. Variety in the food supply for each individual is in fairly direct ratio to the number of fellow-workers who reach new sources of sustenance. Vigor gained by the adults through varied diet and the assimilation of new chemical compositions, would tend to increase the stamina of the growing young through an improved pabulum as well as through heredity.

There is notable difference in the average size and vigor of the individuals in different colonies of ants of the same species.

Relation of Sex and Food to Tenacity of Life.

Male ants shared all tests here presented, but whatever the test undergone, the males showed far less tenacity of life than did either the queens or the workers.

If, as has long been held, the product of unfertilized ant-eggs are males while the product of fertilized ant-eggs are females, then it may be that the absence of certain chemical elements contained in the fertilized egg is a cause of lesser tenacity of life in the male ants.

In all tests of vitality, ants of largest stature among their species showed greatest tenacity of life. Whether the test applied

were endurance of heat,[1] submergence in water, deprivation of food, excision of parts of the body, or administration of poison, the larger the ant, the more probable its survival.

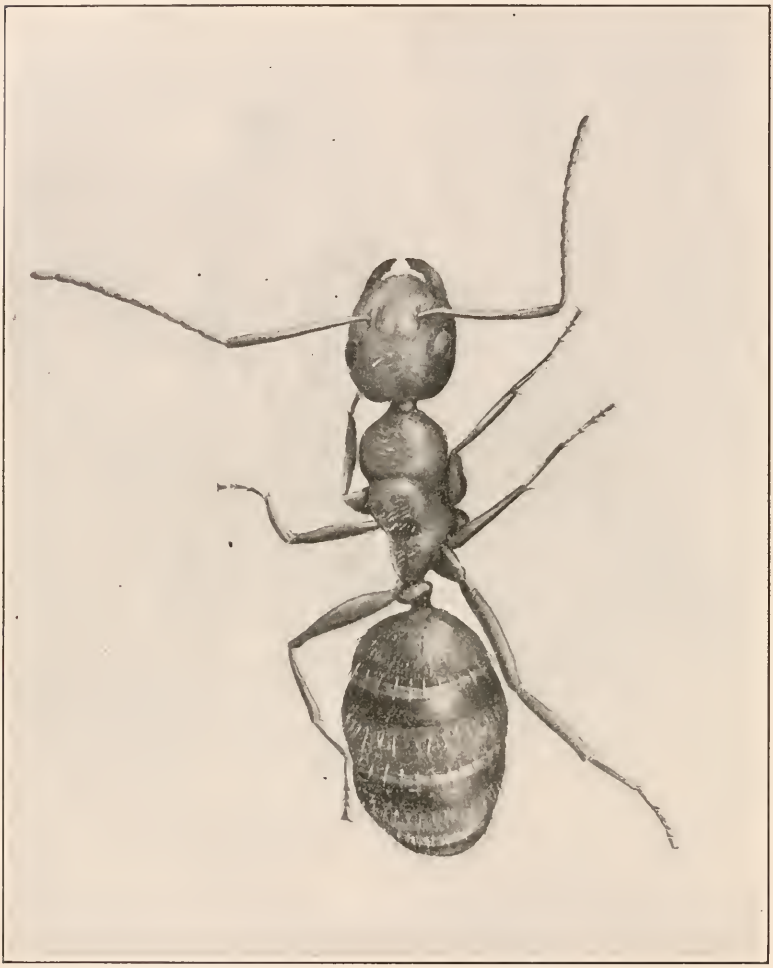


FIG. 1. *Camponotus pennsylvanicus* worker. × 6. From a photograph taken by Mr. J. G. Hubbard and Dr. O. S. Strong, and retouched by Dr. J. H. Macgregor.

There appears to be also a relation between size and natural longevity. Two hundred *Stenamma fulvum piceum* workers,

[1] See paper referred to in previous note, BIOLOGICAL BULLETIN, June, 1904.

majors, minors and minims, were segregated by me in August, 1901, and were kept under observation until August, 1904. At the end of the three years there were eleven survivors, and ten of these were of the largest stature attained by these ants, seven millimeters. Queens, whose longevity probably exceeds that of workers, are ordinarily of larger stature than they.

It has long been known that the size of an ant depends on the quantity and quality of nutriment taken while in the larval stage; but larval nutrition determines not only the size of the ant within the limits of its species; it also determines something of greater influence in the life of the individual and the persistence of the tribe, the probabilities of survival under adverse conditions.